THE PLANT DISEASE REPORTER

Issued By

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 44 Number 7

July 15, 1960

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

COMMONWEALTH MYCOLOGICAL INSTITUTE
9 AUG 1960

SUGGESTIONS FOR PREPARATION OF MANUSCRIPTS FOR THE PLANT DISEASE REPORTER

(1) GENERAL: The Reporter page measures 9 inches long with the heading or 8 3/4 inches for the text part, by 6 inches wide. The copy is typed on a larger page, 11 1/4 inches of text or 12 inches overall in length by 8 inches in width, and reduced 25 percent in the photographic process of reproduction. Illustrations or tables larger in either dimension will take a correspondingly greater reduction. Only one size of type is available for text, footnotes, or tables.

(2) MANUSCRIPTS should be the original ribbon copy, not carbons, clearly typed and double-spaced throughout, including tables, footnotes, and bibliographies. (Note -- only one copy is needed.) Footnotes should be typed at the bottom of the page.

(3) ABSTRACTS are requested for all except very short articles.

(4) CAUSES OF DISEASES should be named. For bacteria, fungi, nematodes, etc., give the Latin name of the organism; for viruses either or both the accepted common name of the virus or a Latin name if you prefer it and there is one; for non-parasitic diseases state the causal factor if it is known. If the cause of a disease has not been determined say so.

(5) LITERATURE REFERENCES should be given in alphabetical order and numbered for citation in the text. We follow the AIBS suggestion of placing the year of publication after the author's name. Please check your references carefully since we cannot do it for you. Be sure that text citations and bibliography agree; that foreign-language references are correct; that number or month is cited for periodicals that are not paged consecutively throughout the volume.

(6) NAMES OF FUNGICIDES should be given according to the suggestion of McCallan et al. in Phytopathology (45 (6): 295-302. 1955).

(7) ILLUSTRATIONS should be sent to us unmounted. To prevent mistakes, write figure numbers on the back, and mark the top of each print when necessary. A sketch can show a preferred arrangement but please keep in mind page size, shape, and standard reduction (see above under General), and remember that figure titles and legends are part of the page. Lettering should be clear and large enough to be legible after reducing. Drawings, maps and graphs can be photographs or originals, but should be finished and ready for reproduction, not just sketches.

(8) TABLES should be carefully thought out with particular attention to the Reporter's limitations in reproduction. Make titles and headings definite and self-explanatory. Designate footnotes in tables with superscript lower-case letters. Be sure that text discussion agrees with the data in the table. Do not abbreviate names of crop varieties.

(9) REPRINTS cannot be supplied since there is no way in which we can be reimbursed. However,

(10) The MULTILITH PLATES from which reprints can be made will be sent if requested at the time the article is submitted. The press size of these plates used for the Reporter is designated as small -- maximum image 9 1/2 by 13 inches, maximum paper size 9 3/4 by 14 inches -- for Model 1250. Most of the Experiment Stations have this type of multilith machine.

ACCEPTANCE OF MANUSCRIPTS

The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to 12-double-spaced typed pages, including tables, graphs, and photographs. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases. By following this procedure we hope to continue publishing all articles promptly.

Paul R. Miller

Manuscripts for and correspondence about this publication should be sent to:

PLANT DISEASE REPORTER
Epidemiology Investigations, Crops Protection Research Branch
Plant Industry Station, Beltsville, Maryland

THE PLANT DISEASE REPORTER

Crops Research Division | Plant Industry Station, Beltsville, Maryland
Volume 44 | July 15, 1960 | Number 7

CONTENTS

1. A new race of bean rust in Maryland
W. J. ZAUMEYER 459

2. Transmission of tobacco ringspot virus on tools during harvesting flowers and corms of gladiolus
PHILIP BRIERLEY and FLOYD F. SMITH 463

3. Evaluation of soil fungicides against Fusarium solani isolated from feeder roots of citrus trees
R. C. CETAS and RODNEY WHIDDEN 465

4. Nematodes associated with the rootlets of western white pine in northern Idaho
W. R. NICKLE 470

5. Seed deterioration as a factor in nub-root production in cotton
A. B. WILES and J. T. PRESLEY 472

6. Reaction of Rhizoctonia solani isolates to certain chemicals
J. B. SINCLAIR 474

7. Nitrogen supplement in the Louisiana-Mississippi river delta as a possible control for Verticillium wilt of cotton
D. C. NEAL and J. B. SINCLAIR 478

8. Effects of soil fumigants on the occurrence of nematodes in field bins
EARL B. MINTON, et al. 479

9. Preliminary studies with DBCP cotton seed treatment for controlling the root-knot nematode
JOHN H. O'BANNON and HAROLD W. REYNOLDS 484

10. Additional characteristics of Panicum mosaic virus
W. H. SILL, Jr. and M. K. DESAI. 487

11. Observations on the natural spread of the so-called peach stunt virus in a California peach orchard
H. KEITH WAGNON, et al. 488

12. Vegetable diseases in North Carolina during 1958 and 1959
N. N. WINSTEAD, et al. 491

13. Variation in maize seedling blight symptoms with changes in pathogen species, isolate and host genotype
GRAYDON C. KINGSLAND and CLIFFORD C. WERNHAM 496

14. Maize synthetics for disease resistance
C. C. WERNHAM 498

15. Insecticide treatments for aphid control in relation to spread of barley yellow dwarf virus
E. A. DICKASON, et al. 501

16. Cercospora bunchosiae, a new leafspot disease of Barbados cherry
HARRY C. BURNETT and J. R. McFARLIN 505

17. Broad spectrum fungicides tested for control of melting-out of Kentucky bluegrass and Sclerotinia dollar spot of Seaside bentgrass
HOUSTON B. COUCH and LAURENCE D. MOORE 506

18. The use of the onion test as a quantitative method for determining the distribution of emulsifiable DBCP in soil
A. RINKOV, et al. 510

19. Spring dead spot of bermudagrass
D. F. WADSWORTH and H. C. YOUNG, Jr. 516

20. Studies on the Phytophthora rot of sugarcane seed pieces in Louisiana
T. van der ZWET, et al. 519

21. Use of herbicides to break the life cycle of the bentgrass nematode, Anguina agrostis (Steinbuck 1799) Filipjev 1936
WALTER J. APT, et al. 524

22. Needle blight of redcedar, Juniperus virginiana L.
ARTHUR KELMAN, et al. 527

23. A simple and inexpensive tractor-mounted inoculum applicator
WAYNE R. SITTERLY and GUY L. BUCKNER, Jr. 532

24. Physalospora obtusa (Cke.) Winter on South Carolina live oaks
WAYNE R. SITTERLY 533

25. Relationship between injury by the clover root curculio and incidence of Fusarium root rot in Ladino white clover
J. H. GRAHAM and R. C. NEWTON 534

26. The occurrence of chlorogenic acid in strawberry plants affected with crinkle and yellows viruses
RICHARD D. DURBIN, et al. 536

27. Differentiation of oat Helminthosporia by the ragdoll method
S. S. IVANOFF 538

28. Phytophthora root rot of soybeans as affected by soil temperature and Meloidogyne hapla
THOMAS D. WYLLIE and DONALD P. TAYLOR 543

29. A genetic abnormality in an Idaho clone of Fragaria vesca
A. W. HELTON 546

30. A strain of tobacco mosaic virus associated with a wilt and stem necrosis of mature, field-grown bell pepper
HARRY H. MURAKISHI and SHIGEMI HONMA 550

31. Effect of soil fungicides upon soil-borne plant pathogenic bacteria and soil nitrogen
DONALD E. MUNNECKE and JOHN FERGUSON 552

32. Watermelon disease incidence in central Florida, 1931-1959
N. C. SCHENCK 556

33. Toxicity of PCNB to Magnolia fuscata
NATALE ZUMMO and A. G. PLAKIDAS 559

34. Evaluation of Phaltan for the control of grape diseases
E. F. TASCHENBERG and A. J. BRAUN 560

35. Trunk and branch canker of coffee trees in Guatemala
WILLIAM C. SNYDER, et al. 566

36. Recent occurrence of bacterial blight of poinsettia in Florida
LORNE A. McFADDEN and DON B. CREAGER 568

37. The production and use of zoospore suspensions of Phytophthora spp. for investigations on diseases of citrus
L. J. KLOTZ and T. A. DeWOLFE 572

38. A comparison of polyethylene plastic bags and glass jars as incubation chambers for obtaining nematodes from roots
A. C. TARJAN 574

39. Winter outbreaks of citrus brown rot in Florida
MORTIMER COHEN and L. C. KNORR 578

40. Woody galls on citrus associated with vein-enation virus infection
JAMES M. WALLACE and R. J. DRAKE 580

41. The apothecial stage of Botrytis squamosa, cause of tip and leaf blight of onions
D. M. McLEAN 585

42. Bacterial spot resistance of introduced peppers
GROVER SOWELL, Jr. 587

43. Modification and adaptation of Popp's technique to routine detection of Ustilago nuda (Jens.) Rostr. in barley embryos
T. KAVANAGH and D. L. MUMFORD 591

44. An easily constructed clip-type insect cage
JAMES W. GUTHRIE and GUY W. BISHOP 592

45. Another screened immersion plate for isolating fungi from soil
FRANCIS A. WOOD and ROY D. WILCOXSON 594

46. Powdery mildew of potato in Argentina
R. E. PONTIS and J. M. FELDMAN 595

47. A high incidence of Diaporthe phaseolorum occurring in the seed of soybeans from southwestern Ontario
V. R. WALLEN 596

48. A new record of violet scab (Sphaceloma violae)
ANNA E. JENKINS 596

49. Book Review (Diseases and Pests of Ornamental Plants)
PAUL R. MILLER 597

A NEW RACE OF BEAN RUST IN MARYLAND

W. J. Zaumeyer[1]

Summary

A new race of bean rust, highly infectious to bush snap beans but not to most pole and dry bean varieties, was isolated from beans growing in two locations in Maryland in 1959. Of 56 bush snap bean varieties inoculated, 5 were homozygous resistant, only one, Tennessee Green Pod, was immune, and 5 were heterozygous. Of the 19 pole varieties tested, 15 were immune or highly resistant, 2 were heterozygous, and 2 were homozygous susceptible. Sixteen of the 18 dry bean varieties inoculated were immune. The outstanding differences between the new race and the previously reported ones are that the former infects most of the bush snap bean varieties which are tolerant or resistant to the majority of the other races and is not able to infect most pole and dry bean varieties which are infected by the majority of the other races.

Although bean rust caused by Uromyces phaseoli var. typica Arth. has been observed in Maryland from time to time, it was not of concern to bean growers until 1948, when Weaver and Marcus (3) observed it in Kent County, Maryland, in the northern part of the Eastern Shore, where it caused considerable loss in late-summer and early-fall crops of snap beans. The isolate was identified by them as a new race of the rust fungus but was not given a numerical designation.

In 1952 Fisher (1), in his studies on new physiological races of bean rust, reported the Maryland race as identical with his race 28 which he collected in Maryland.

In 1958 bean rust again appeared in the State and heavy losses to snap beans resulted in Somerset County in the vicinity of Princess Anne, in the southern part of the Eastern Shore. It was so destructive there that many bean plantings were abandoned before harvest. No effort was made to identify the race of the rust in question.

In 1959 the disease was again widespread in fall-grown beans in this area as well as in the vicinity of Frederick, Maryland, about 175 miles northwest of Princess Anne. Here the disease was observed first in mid-summer and became very severe in late-summer and fall-grown crops. Many bean fields were so seriously affected that the crops were not harvested.

Prior to these outbreaks, bean rust was not a problem in the Eastern and Northeastern States except in certain small areas in Virginia where losses as high as 10% were reported about 30 years ago. The disease was occasionally observed in both New York and the New England States in dry bean varieties in the fall but no damage was reported. Snap beans were seldom affected although about 15 years ago considerable damage was reported to a small acreage of Blue Lake pole beans being grown experimentally in New York. Bush varieties of snap beans growing in the same area were not affected.

EXPERIMENTAL RESULTS

Spores from infected beans of several varieties growing in the vicinity of Frederick and Princess Anne, Maryland, inoculated to the differential bean varieties used for identifying races of bean rust showed that the same race was responsible for the losses in both locations. It differed, however, from any race heretofore reported. Table 1 shows that the new race, designated 32, is similar to race 28, previously reported by Fisher (1) from Maryland, and especially similar to race 10 reported by Harter and Zaumeyer (2), from Florida in 1941. The difference in reaction of Golden Gate Wax (Table 1) to race 28 and to the new race clearly differentiates them.

As indicated in Table 1, Golden Gate Wax was not inoculated with race 10. When this race was described the variety was not available. While the studies here reported were being conducted, race 10 was not available. Besides the slight differences noted in the reactions of the differential varieties to the new race and to race 10, other varietal differences were observed. String-

---

[1] Principal Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

Table 1. Reaction of the differential bean varieties to several races of bean rust.

Differential variety	Infection grade[a] produced by rust race 10	28	32
U.S. No. 3	3	0	1/3[b]
Bountiful	9	-	10
No. 643	1	0	0
Pinto	2	0	0
No. 765	1	1	0
No. 780	1	2	1
No. 814	0	0	0
Golden Gate Wax	-	0	3-4 / 10

[a]Infection grades range from 0 for immunity to 10 for highest degree of susceptibility. A rating below 5 is considered field resistance.

[b]Numerator indicates size of pustule on upper leaf surface and denominator on lower surface.

less Green Refugee, McCaslan, and Red Kidney varieties were highly resistant to the new race but very susceptible to race 10. Michelite and Robust were immune from infection by the new race but showed 2 and 3 reactions, respectively, to race 10.

Another difference between the new race and the previously described ones is the size of the pustules produced on the lower and upper leaf surfaces of several varieties. The new race produces much larger pustules on the lower than on the upper surfaces of Extender, Golden Gate Wax (Table 1), Potomac, Sensation Refugee 1066, Topcrop, and several other varieties. The other races produce pustules of about the same size on both leaf surfaces. On some varieties the necrotic lesions are as large on the upper as on the lower surfaces, but the sporulation on the upper surfaces is more sparse than on the lower (Fig. 1, A and B).

VARIETAL RESPONSES

Ninety-three varieties of bush- and pole-type snap beans and dry beans (Table 2) were inoculated with a spore suspension of the new race. Only five green-podded bush snap bean varieties were homozygous resistant. These were Stringless Green Refugee, which gave a 2 reaction, U.S. 5 Refugee and Seminole a 1/3 reaction, Tenderwhite a 3/4 reaction, and Tennessee Green Pod immune (Fig. 1, C).

Several varieties were heterozygous in their response; some plants showed a high degree of resistance while others were very susceptible. The following varieties reacted in this manner: Extender, Green Crop, Sensation Refugee 1066, and State Half Runner.

All the wax varieties except Brittle Wax were homozygous susceptible (Table 2). Both immune and susceptible seedlings of this variety were observed.

Table 2. Reaction of bean varieties to the new race of rust.

Class	Varieties Tested	Homozygous resistant	Susceptible[a]
Bush snapbeans:			
Green-podded	47	5	42
Wax-podded	9	0	9
Pole snapbeans:			
Green-podded	18	15	3
Wax-podded	1	0	1
Field or Dry	18	18	0

[a]Includes both homozygous- and heterozygous-susceptible varieties.

It is of interest that among the 19 pole varieties tested 15 were immune or highly resistant and 3 showed a heterozygous reaction; only one of these contained plants with ratings greater than 5, and 2 were homozygous susceptible. All 18 dry bean varieties inoculated were resistant;

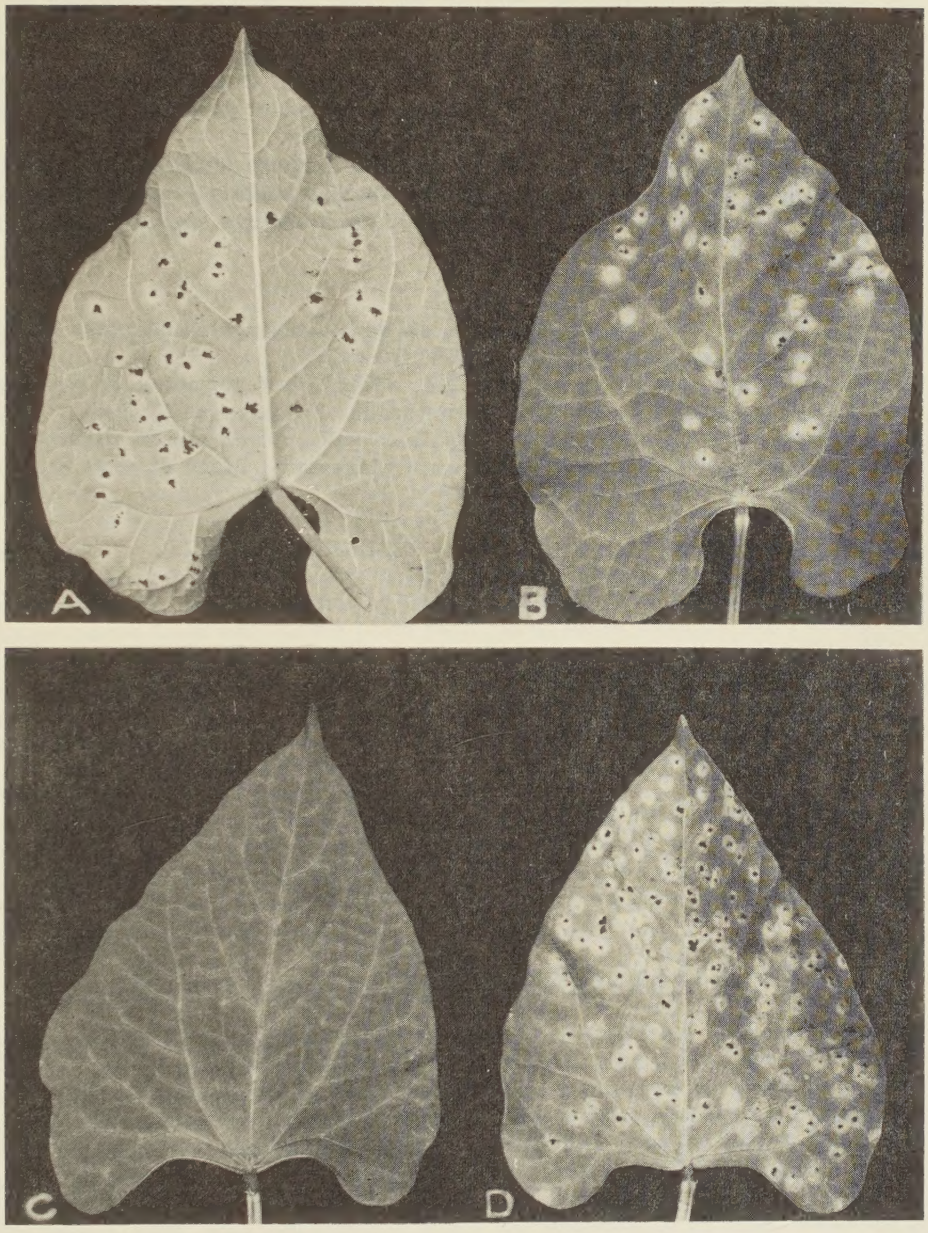


FIGURE 1. Sporulation of the new race of rust on the lower (A) and the upper (B) leaf surface of Commodore bean. Tennessee Green Pod (C) showing immune reaction compared with Topcrop (D) showing a 10 reaction to the new race.

16 of them were immune.

The detailed reactions of the 93 varieties tested and the infection rating of each variety are not recorded in this paper. A table showing the results of the tests has been mimeographed and copies of it can be obtained from the author.

The importance of the new race of rust in the snap bean-producing areas of Maryland and other South-Atlantic States should not be underestimated. All snap bean varieties now being grown in Maryland by processors and market gardeners, except Tenderwhite, are susceptible. These include Bountiful, Contender, Corneli 14, Harvester, Kinghorn, Pearlgreen, Processor, Puregold, Slendergreen, Tendercrop, all strains of Tendergreen, and Topcrop (Fig. 1, D). Of particular interest is that the new race infects a much higher percentage of green- and wax-

podded bush snap bean varieties than of pole and dry bean varieties. Practically all the other previously reported races infected most of the pole and dry bean varieties and relatively few of the bush-type beans. If breeding for disease resistance should become necessary, several bush-type varieties possessing resistance to at least two virus diseases and having excellent horticultural characteristics could be used as the rust-resistant parents. These are Brittle Wax, Extender, Seminole, U.S. 5 Refugee, and Tenderwhite.

Both sulfur, manganese ethylene bisdithiocarbamate (maneb), and zinc ethylene bisdithiocarbamate (zineb) used as a protective spray or dust should readily control the disease. It is doubtful whether control would be successful if the materials were applied as eradicants.

Literature Cited

1. FISHER, H. H. 1952. New physiologic races of bean rust (Uromyces phaseoli typica). Plant Disease Reptr. 36: 103-105.
2. HARTER, L. L., and W. J. ZAUMEYER. 1941. Differentiation of physiologic races of Uromyces phaseoli typica on bean. J. Agr. Research 62: 717-782.
3. WEAVER, L. O., and C. P. MARCUS, Jr. 1949. A new strain of rust caused losses to beans on the Eastern Shore of Maryland. Plant Disease Reptr. 33: 483-484.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE,
UNITED STATES DEPARTMENT OF AGRICULTURE

TRANSMISSION OF TOBACCO RINGSPOT VIRUS ON TOOLS DURING HARVESTING FLOWERS AND CORMS OF GLADIOLUS

Philip Brierley and Floyd F. Smith[1]

Abstract

The tobacco ringspot virus was transmitted from gladiolus to gladiolus on tools in harvesting flowers (1.8%) and in cutting tops preparatory to harvesting corms (10%, 1.9%). This virus was symptomless in the gladiolus cultivars and seedlings used in these tests.

In January 1955 (4) we thought we had evidence that the green peach aphid Myzus persicae (Sulz.) transmitted the tobacco ringspot virus (TRspV), together with the virus of bean yellow mosaic (BV2), from gladiolus to gladiolus. Later in the same year we attempted to duplicate this finding in Crotalaria spectabilis, a seed-propagated plant that responds to mechanical inoculation with distinctive symptoms for each of these viruses. However, from Crotalaria infected with both viruses the green peach aphid regularly transmitted BV2 only, never TRspV. This suggested that our test clon of gladiolus had become contaminated with TRspV as a result of some practice of growing the crop, such as cutting the tops before harvesting the corms, or cutting flowers.

Although previous tobacco indexing of this clon revealed no virus, in September 1957 we re-indexed 90 plants by sap inoculation to tobacco, snapdragon, and Gomphrena globosa. Inoculum was prepared from two leaves from each plant; these were cut with a flamed knife to eliminate danger of further interplant contamination. Snapdragon detected TRspV in 19, tobacco in 15, and Gomphrena in nine plants. In all, 20 plants (22%) proved infected with TRspV, but no other virus was detected. This clon had been propagated in a greenhouse for 12 years with an average of one crop a year. Contamination probably resulted from the practice of cutting away the tops of all glads before harvesting the corms. In some seasons TRspV-infected stock must have been cut just before the seedling clon.

It is customary to use cucumber seedlings to detect TRspV, but in our trials young Marketer cucumber plants, inoculated on the cotyledons, proved no more sensitive to infection than snapdragon. For index purposes the snapdragon has an advantage over cucumber in that plants remain longer in condition for use; snapdragons respond satisfactorily even after cutting back if fertilized to maintain active growth.

Transmission of TRspV by Cutting Tops Before Harvesting Corms

In November 1957, 3 to 4 weeks after the plants had flowered, 20 healthy gladiolus plants were exposed to infection by contaminated shears in removing tops preparatory to harvesting the corms. A TRspV-infected stem was cut with shears immediately before each healthy plant was cut about 1 inch above the soil line. When cut the healthy stems were relatively dry and no accumulation of sap on the shears was evident. When the corms of these plants were harvested there was regrowth of the youngest leaf from the cut stem in 6 of the 20 plants, indicating that growth may still occur at the stage when the corms are harvested. Growth from these corms, replanted in March 1958, was indexed on tobacco and snapdragon in June, when the plants were in flower. Two of 20 plants (10%) yielded TRspV, one in snapdragon only, and one in both test species. Four of the original source plants yielded TRspV when rechecked. All these isolates were confirmed as TRspV by cross protection tests against ATCC No. 98.

In June 1958 a flat of gladiolus seedlings about 1 year from seed, grown in a greenhouse, and not yet propagated, served for additional tests. Twenty-five seedlings selected at random and indexed on snapdragon yielded no virus. The flat was then divided with a wooden partition into two approximately equal parts. Tops were then cut from the control half flat with a flamed knife. In the other half flat each of the seedling tops was cut immediately after cutting a TRspV source plant, at a time when about half the seedling stock was mature and dry.

The seedlings exposed to contamination at harvest were indexed by sap inoculation to snapdragon in February and March 1959. Three plants of 155 (1.9%) were found infected with TRspV;

[1]Pathologist, Crops Research Division, and Entomologist, Entomology Research Division, respectively, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.

none of these three showed symptoms. To eliminate the possibility that seed-borne virus might be involved, 100 control plants were indexed similarly, but no TRspV was detected in these. The small size of the seedling tops when cut, together with the fact that about half were dried out, may account for the low transmission in this test.

Transmission of TRspV by Cutting Flowers

In May 1959, 57 seedlings were cut with a sharp knife as the first floret opened, the knife passing through a TRspV-infected gladiolus immediately before cutting each seedling. These seedling plants were indexed on snapdragon in March 1960. Only one of 57 plants proved infected (1.8%) and this plant also was symptomless.

DISCUSSION

Walters (5, 6) first demonstrated transmission of TRspV by the grasshopper Melanoplus differentialis (Thos.) from tobacco to tobacco. Shepherd and Struble (3) transmitted TRspV from watermelon to watermelon with this grasshopper. Dunleavy (1) showed that M. differentialis and two other grasshoppers could transmit TRspV from tobacco to soybean and from soybean to soybean. In all three studies grasshoppers proved relatively inefficient as vectors of TRspV. No one has yet demonstrated that grasshoppers can transmit TRspV to gladiolus, but it seems likely that these insects are responsible for the original introduction of this virus into this crop. However, other means of transmission must take place to account for the high frequency of TRspV attained in gladiolus.

A group of Florentine gladiolus were examined on the Plant Industry Station in 1958. Short plants with white-streaked leaves and bleached bracts were considered possibly affected with stunt or white break, but diagnosis in this white-flowered variety proved difficult. Regrown in a greenhouse in 1959, samples of these Florentine gladiolus were found 100% affected with BV2, 59% with TRspV, and 33% with cucumber mosaic virus (CV). Leaf mottling only accompanied BV2 infection. No symptoms specific for TRspV were recognized. Plants infected with CV averaged 36% shorter than those infected with BV2 only; their leaves were streaked with white, the bracts streaked or mottled rather than uniformly bleached as noted in the field, and the flowers deformed and usually unsalable. Thus, of the three viruses present, CV alone was clearly damaging to this variety. Injury by the greenhouse fumigant TEPP sometimes simulates the white streaking in leaves but not the dwarfing or flower-deforming symptoms of CV.

In commercial practice (2) gladiolus stems are cut a few inches above the soil in harvesting flowers. It is customary also to top the plants before digging the corms. Certain machines (2) designed to cut the tops below ground should be partly self-cleaning, but machines cutting the tops above the soil should be effective in transmitting TRspV. The commercial practice of harvesting corms before the tops die also favors transmission of TRspV.

Literature Cited

1. DUNLEAVY, JOHN M. 1957. The grasshopper as a vector of tobacco ringspot virus in soybean. Phytopathology 47: 681-682.
2. MAGIE, ROBERT O., and W. G. COWPERTHWAITE. 1954. Commercial gladiolus production in Florida. Florida Agr. Exp. Sta. Bull. 535. 67 pp.
3. SHEPHERD, ROBERT J., and F. BEN STRUBLE. 1956. Tobacco ringspot virus on watermelon. Phytopathology 46: 358-362.
4. SMITH, FLOYD F., and PHILIP BRIERLEY. 1955. Aphid transmission of tobacco ringspot virus in gladiolus. Plant Disease Reptr. 39: 35.
5. WALTERS, H. J. 1951. Grasshopper transmission of three plant viruses. Science 113: 36-37.
6. WALTERS, H. J. 1952. Some relationships of three plant viruses to the differential grasshopper, Melanoplus differentialis (Thos.). Phytopathology. 42: 355-362.

CROPS RESEARCH AND ENTOMOLOGY RESEARCH DIVISIONS,
AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT
OF AGRICULTURE

EVALUATION OF SOIL FUNGICIDES AGAINST FUSARIUM SOLANI ISOLATED FROM FEEDER ROOTS OF CITRUS TREES

R. C. Cetas[1] and Rodney Whidden[2]

Abstract

Preliminary soil fungicide-screening tests were conducted against Fusarium solani (Mart.) Appel & Wr. emend Snyd. & Hans. in percolation tubes. DAC-649, nabam, and Vapam were the most effective fungicides tested.

Spreading decline is one of the most important diseases of citrus trees in Florida. Work on the nature and cause of the disease has shown that the burrowing nematode, Radopholus similis (Cobb) Thorne, is the primary causal agent (8). The burrowing nematodes penetrate the feeder roots in the region of cell elongation and root hair formation. No wound cork or other protective barrier is formed in the healthy tissues surrounding the cavities that result from the feeding activities of the nematode (2). Consequently, the roots are susceptible to attack by wound pathogens following penetration and a number of bacteria, fungi, and other microorganisms have been found associated with the feeder roots of decline-affected trees (7, 8, 9).

Feldmesser and coworkers (3) attempted to eliminate fungi as a possible cause of symptoms of spreading decline by treating small, decline-affected Duncan grapefruit seedlings with drenches of captan in a greenhouse experiment. They obtained positive growth responses with the drench treatments and the treatments had no effect on the number of nematodes, Radopholus similis and Pratylenchus, recovered from the roots. They concluded that some factor other than nematodes was controlled but did not determine that factor.

The work presented here was a preliminary search for fungicides that could be used for the control of one or more of the secondary fungi that have been found associated with the feeder roots of citrus trees affected with spreading decline. Since various species of Fusarium have been isolated more frequently than species of other genera[3] (8), a representative of this genus was chosen for the test organism. Fusarium solani (Mart.) Appel & Wr. emend Snyd. & Hans.[4] isolated from burrowing nematode-infected rootlets of spreading decline-affected citrus trees was selected because similar work has been conducted with F. oxysporum f. cubense (5). The equipment used in these investigations is shown in Figure 1.

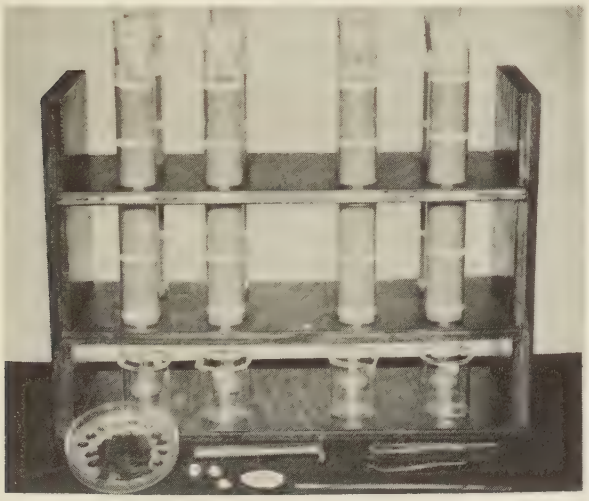


FIGURE 1. Equipment used in fungicide-screening tests. (Photograph by G. R. Grimm).

[1]Plant Pathologist, United States Department of Agriculture, Agricultural Research Service, Crops Research Division, Orlando, Florida on sabbatical leave from the Department of Plant Pathology, Cornell University, Long Island Vegetable Research Farm, Riverhead, New York.
[2]Biological Aid, United States Department of Agriculture, Agricultural Research Service, Crops Research Division, Orlando, Florida.
[3]Unpublished data.
[4]Identification confirmed by W. C. Snyder, University of California, Berkeley, California.

PROCEDURE

In one group of experiments, agar discs were cut from 12- to 14-day-old sporulating Petri dish cultures of Fusarium solani. These were placed 2, 4, 6 and 8 inches below the soil surface in percolation tubes (5) filled with autoclaved Eustis fine sand. In some experiments a fifth disc was placed 0.5 inch below the soil surface. The agar discs were 1 cm in diameter and 2 to 4 mm thick. They were cut with a flamed sterilized cork borer and mounted on sterilized straight pins. To prevent the agar discs from falling off the pins, a filter paper disc was placed on the pin before the agar disc was mounted. The nonsporulating surface of the agar disc was always toward the filter paper. After the 10 x 1 3/16-inch (inside diameter) percolation tubes were filled to each level, the sand was packed by dropping the rack about 4 inches onto the laboratory bench five times. The agar discs were then dropped into the tubes. All tubes were filled to within 1 inch of the top. F. solani was grown on PDA (potato-dextrose agar). The water suspension or solution of the candidate fungicide(s) was poured into the designated tubes until a few ml leached from the bottom. Fifty to 60 ml was required to saturate. Soil columns drenched with water served as controls. The soil columns were supported by saran screen stapled to the bottom of the middle shelf of the rack (Fig. 1). A filter paper disc about 1 5/16 inches in diameter was put on the screen to prevent loss of sand from the bottom of each tube. A straight pin through the center of these discs directed the leachings into the beakers below. Unless otherwise indicated, the wet soil after standing 24 hours was carefully removed with a flame-sterilized stainless steel spatula. As the agar discs and pins were exposed, they were carefully removed from the soil with sterile forceps. Excess soil was removed by lightly tapping the forceps on the rim of the dish into which the saturated soil was emptied. The agar discs were then separated from the filter paper, removed from the pin, and plated on PDA that contained 100 μg of novobiocin per ml (1). Records on survival of F. solani were taken 5 to 7 days later.

In a second group of experiments, the percolation tubes were filled to within 1 inch of the top with autoclaved Eustis fine sand artificially infested with F. solani. The soil was packed by dropping the rack of filled tubes on the laboratory bench 20 times. The soil was drenched with the candidate fungicides as described for the first group of experiments. After standing for 24 hours, the saturated soil was carefully removed from the percolation tubes. At predetermined levels, a small crumb of soil was removed with a sterile full spear dissecting needle and placed in a sterile Petri dish. When the soil crumbs were dry, they were broken with a sterile scalpel and approximately 15 ml of PDA that contained 100 μg of novobiocin per ml was poured into each dish. Three or four days later the colonies of F. solani were counted and recorded. The artificially infested soil was prepared by mixing two 100-ml beakers full of yellow corn meal with 4 liters of steam-pasteurized Eustis fine sand. Two-liter Erlenmeyer flasks were filled with the mixture and autoclaved for 3 hours at 15 p.s.i. The flasks of soil were inoculated with a spore suspension of F. solani 24 hours later. When the soil cultures were about 4 weeks old, the soil was emptied into a clean vessel and thoroughly mixed. The soil was put into the percolation tubes immediately.

A third group of experiments was conducted in a manner similar to the second group except that Eustis fine sand from the vicinity of roots of citrus trees affected with spreading decline was used.

All experiments were conducted in the laboratory at 68° to 74° F. Before drenching the soil moisture varied between experiments from 1.5 to 6.0%, based on the wet weight of the soil, but was the same for all columns in a given experiment.

RESULTS

In preliminary experiments, PCNB[5], both in the wettable powder and emulsifiable concentrate formulations, at dosages of 200 ppm active in the drench solutions had no effect on the growth of F. solani from the agar discs recovered from the 2-inch depth or deeper in the percolation tubes. Vapam[6] at the rate of 200 ppm active did kill the fungus at the 2-inch level, and after 7 days only a trace of mycelial growth was observed on the agar discs recovered from the 4-, 6-, and 8-inch depths.

---

[5]Pentachloronitrobenzene; available as Terraclor 75% wettable powder and as Terraclor 24% emulsifiable concentrate, Olin Mathieson Chemical Corporation.

[6]31% sodium methyldithiocarbamate (anhydrous), Stauffer Chemical Company.

Table 1. Survival of Fusarium solani on agar discs[a] after 24 hours in soil drenched with different concentrations of selected fungicides.

Fungicide and concentration (ppm)[b]	: Survival readings[c] for indicated depth (in inches) 2	4	6	8
DAC-649[d]: 0	2.0	2.0	2.0	2.0
12.5	2.0	1.4	2.0	2.0
25	1.4	1.0	1.4	2.0
50	0.5	0.5	0.9	1.8
100	0.0	0.0	0.5	0.8
200	0.0	0.0	0.0	0.2
Nabam[e]: 0	2.0	2.0	2.0	2.0
25	1.8	1.9	2.0	2.0
50	0.4	0.0	1.6	2.0
100	0.0	0.0	0.5	1.9
200	0.0	0.0	0.0	1.8
500	0.0	0.0	0.0	0.7
Captan[f]: 0	2.0	2.0	2.0	2.0
250	2.0	2.0	2.0	2.0
500	2.0	2.0	2.0	2.0
1000	1.9	2.0	2.0	2.0
2000	1.7	2.0	2.0	2.0
4000	1.5	2.0	2.0	2.0

[a]Sporulating agar disc, 1 cm in diameter, was placed at the indicated depth in percolation tubes filled with autoclaved Eustis fine sand and drenched with indicated concentration of the fungicide.
[b]Concentration of active ingredient in the drench solution or suspension.
[c]Survival readings were made 5 to 7 days after the agar discs were plated on PDA that contained 100 μg of novobiocin per ml. 0 indicates no mycelial growth; 1 mycelial growth was retarded, about one-half that of the controls; 2 no effect on growth of mycelium.
[d, e, f]. See footnotes 7, 8 and 9 of text.

F. solani was killed at various levels with drenches that contained 100 ppm or more of DAC-649[7] or of nabam[8] when sporulating agar discs were buried in the percolation tubes. Mycelial growth was reduced only slightly from agar discs recovered from the 2-inch level in soils drenched with suspensions that contained 1000, 2000, and 4000 ppm of captan[9] (Table 1). When drenches of these fungicides were applied to artificially infested soil, DAC-649 was effective at lower dosages and at greater depths than was nabam. Nabam was more effective than captan (Table 2). When soil from around roots of citrus trees affected with spreading decline was drenched with various dosages of captan, the number of fungus colonies recovered from crumbs of soil was reduced considerably (Table 3). The majority of these colonies were various species of Aspergillus and of Penicillium. A few colonies of Fusarium appeared on each plate and the number of Fusarium colonies was not reduced as the dosage of captan was increased from 0 to 200 ppm.

Since a single drench of 4000 ppm of captan did not kill F. solani on agar discs at the 2-inch depth (Table 1), two experiments were conducted to determine what effect, if any, additional water drenches have on the downward movement of captan as indicated by the survival of F. solani in agar discs buried at various depths in the percolation tubes. In these experiments the soil in the percolation tubes was saturated with 200 and 2000 ppm suspensions of captan. Fifty ml of water, enough to displace most of the free moisture in the soil in the percolation tubes, was poured into each tube once each day until the designated number of drenches had been applied. The agar discs were recovered from all tubes 15 days after the initial drenches were applied. When the experiment was discontinued, mycelium of F. solani

[7]3,3,4,4,-tetrachlorotetrahydrothiopene-1,1-dioxide; formulated as a 50% wettable powder, Diamond Alkali Company.
[8]Disodium ethylenebis(dithiocarbamate); formulated as a 22% solution, Dithane D-14, Rohm & Haas Company.
[9]N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide; formulated as a 50% wettable powder, California Spray-Chemical Corporation.

Table 2. Survival of Fusarium solani after 24 hours in artificially infested soils drenched with various concentrations of selected fungicides.

Fungicide and concentration (ppm)[a]	Average number of colonies per crumb of soil from indicated depth (in inches)[b]				
	Surface	2	4	6	8
DAC-649[c]: 0	163	222	200	208	190
12.5	33	180	156	246	246
25	2	4	4	8	172
50	1	1	0	1	25
100	0	1	0	1	0
200	0	1	1	1	1
Nabam[d]: 0	116	128	156	136	154
25	57	116	186	207	188
50	12	9	112	174	196
100	0	1	3	111	184
200	1	1	0	74	187
500	0	0	1	12	40
Captan[e]: 0	198	292	286	174	202
50	142	296	290	326	232
100	105	316	306	306	230
200	60	232	260	290	350
500	10	138	378	214	266
1000	3	174	330	336	354
2000	3	86	304	244	304

[a]Concentration of active ingredient in drench solution or suspension.
[b]Crumbs of soil were plated on PDA that contained 100 μg of novobiocin per ml. The colonies of F. solani were counted 4 days after the soil crumbs were plated. Figures are the average number of colonies counted on one plate in each of two replications.
[c, d, e] See footnotes 7, 8 and 9 of text.

Table 3. Survival of all fungi[a] after 24 hours in "spreading decline"-soil drenched with various dosages of captan.

Fungicide concentration (ppm)[b]	Average number of fungus colonies per crumb of soil from indicated depth (in inches)[c]		
	1.5	4.5	7.5
0	183	325	289
12.5	108	217	310
25	88	103	285
50	63	159	220
100	57	110	212
200	62	103	120

[a]All fungi in the soil that grew on PDA that contained 100 μg of novobiocin per ml.
[b]Concentration of active ingredient in drench suspension.
[c]Colonies were counted 4 days after the crumbs of soil were plated. Figures are the average number of colonies on two plates in each of two replications.

was growing out of the bottom of all percolation tubes and it was recovered from the filter paper discs placed on the saran screen to support the soil columns.

When the initial drench contained 200 ppm of captan, drenching the soil with water on three or more consecutive days killed or drastically retarded the growth of F. solani from the agar discs buried 0.5 inch below the surface but had no effect on the growth of the fungus from discs recovered from 2 or more inches deep. F. solani was not recovered from the agar discs buried 0.5 inch deep in the soils drenched with suspensions that contained 2000 ppm of captan. The use of the additional water drenches retarded the growth of the fungus from the agar discs recovered from the 2-inch level of the soils drenched five or more times with water following the initial drench of 2000 ppm of captan. In contrast, the additional water drenches had no effect on the recovery of F. solani from agar discs buried 4, 6, and 8 inches deep in the percolation tubes.

DISCUSSION

Of the fungicides tested DAC-649, nabam, and Vapam showed the most promise for control of F. solani. Unfortunately, time did not permit determination of the effect of the fungicides on the growth of citrus seedlings.

The active ingredient of DAC-649 apparently acts as a fumigant, because when an effective dosage was used the results were similar at all levels between 0 and 6 inches. In contrast, each dosage of nabam was less effective at the lower levels than at the upper levels (Table 2). The explanation for the decrease in the effectiveness of the nabam solutions was a gradual decrease in concentration as the drench solution leached through the soil. Undoubtedly this was accomplished 1) by dilution with the free moisture in the soil, 2) by diffusion of nabam into the bound water on the soil particles, and 3) by chemical reactions between nabam and other chemicals in the soil. These results indicate that nabam solutions do not have any fumigation action. Therefore, the liquid phase must come in contact with the fungus before it is effective.

Captan is only slightly soluble in water and the results of these experiments indicate that most of it probably remains in the top 2 inches of soil when it is applied as a drench. A comparison of results in Table 2 and Table 3 indicates that other soil fungi are more sensitive to captan than is F. solani. Some of the more sensitive fungi are species of Aspergillus and of Penicillium.

The results of the work conducted by Newhall (5) and of the experiments reported here show that the application of captan as a drench will not control F. oxysporum f. cubense and F. solani below the 2-inch depth in the soil. The growth responses obtained by Feldmesser and coworkers (3) may have been due to the control of the Fusaria by captan leached through the soil by repeated watering or to the fact that captan or one of its degradation products was absorbed by the plant and acted as a systemic fungicide (4, 6) or as a growth regulator.

Literature Cited

1. BUTLER, E. E., and R. B. HINE. 1958. Use of novobiocin for isolation of fungi from soil. Soil Science 85: 250-254.
2. DuCHARME, E. P. 1959. Morphogenesis and histopathology of lesions induced on citrus roots by Radopholus similis. Phytopathology 49: 388-395.
3. FELDMESSER, J., R. V. REBOIS, and A. L. TAYLOR. 1959. Progress report on growth responses of burrowing nematode infected citrus following chemical treatments under greenhouse conditions. Plant Disease Reptr. 43: 261-263.
4. MAY, CURTIS, JOHN G. PALMER, and EDWARD HACSKAYLO. 1958. Inhibition of growth of Ceratocystis ulmi in vitro by residues from extracts of soils and of plants growing in soils treated with captan or Orthocide 50W. Plant Disease Reptr. 42: 696-702.
5. NEWHALL, A. G. 1958. An improved method of screening potential soil fungicides against Fusarium oxysporum f. cubense. Plant Disease Reptr. 42: 677-679.
6. ROSEN, H. R. 1959. Preliminary report on the systemic activity of captan when applied to the soil for the control of black spot of roses. Plant Disease Reptr. 43: 1176-1177.
7. SHERBAKOFF, C. D. 1953. Fusaria associated with citrus feeder roots in Florida. Phytopathology 43: 395-397.
8. SUIT, R. F., and E. P. DuCHARME. 1953. The burrowing nematode and parasitic nematodes in relation to spreading decline of citrus. Plant Disease Reptr. 37: 379-383.
9. SUIT, R. F., E. P. DuCHARME, H. W. FORD, and T. L. BROOKS. 1953. Nature, cause and control of citrus decline. Ann. Rept. Florida Agr. Exp. Sta. 1953: 188-190.

FRUIT AND NUT CROPS RESEARCH BRANCH, CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, ORLANDO, FLORIDA

NEMATODES ASSOCIATED WITH THE ROOTLETS OF WESTERN WHITE PINE IN NORTHERN IDAHO[1]

W. R. Nickle[2]

Summary

Several genera of plant parasitic nematodes have been found in soil and root samples taken throughout the white pine type in northern Idaho. This investigation was carried out to provide information pertaining to a disease of western white pine known as pole blight. Female nematodes of the genus Heterodera have been found embedded in white pine rootlets taken from a diseased stand. These forms appeared destructive to the mycorrhizal rootlets. Observations and preliminary tests indicate that western white pine is a host of Trichodorus elegans Allen. This stubby-root nema has been found in all pole-blighted areas sampled and was not generally found in stands which did not exhibit pole blight symptoms. Other plant parasitic nematodes, belonging to the genera Criconemoides, Criconema, Helicotylenchus, and Tylenchorhynchus, have been recovered from soil samples taken from the rhizosphere of diseased white pine trees.

The applied aspects of plant nematology have dealt almost entirely with diseases of agricultural crops, and little attention has been given to the role of nematodes in the soil environment of forest trees. Also, forest pathologists have tended to consider nematodes as being outside their sphere of professional interest. A review of the literature shows that virtually no studies have been made on the nematode parasites as the possible cause of diseases of forest trees other than seedlings. Criconemoides rusticum (Micoletsky) Taylor (3) was found associated with the littleleaf disease of shortleaf pine and was demonstrated to cause a deterioration of the fine rootlets and mycorrhizae of potted pine seedlings. There is evidence that some nematodes are injurious to forest nursery stock (2) and to many cultivated trees such as citrus, peach, and walnut. The present investigation was carried out to provide information pertaining to the pole blight disease of western white pine.

Pole blight is a serious disease of unknown cause that affects over 92,000 acres of pole-sized western white pine (Pinus monticola) in the Inland Empire. Death of these trees usually occurs within 5 to 10 years and is preceded by dieback of the crown and deterioration of the root system (4).

In the course of pole blight investigations over the past 10 years, researchers have found themselves face-to-face with a frustrating lack of basic information on the root environment of western white pine. This study was an attempt to provide some of this information by determining the identity of nematodes present in both healthy and pole-blighted white pine stands in northern Idaho, with special reference to the plant parasitic forms (5).

Soil samples for nematode analyses were taken from pole-sized white pine stands in northern Idaho during the growing seasons of 1955-1958. Twenty-five areas were sampled during the summer of 1957 including 14 pole-blighted stands, 5 healthy stands in the pole blight range, and 6 healthy stands outside of the general range of pole blight. Soil was sampled to the 2-foot level and processed by standard methods. Ten samples were collected from each area.

This initial survey revealed the presence, in diseased areas, of plant parasitic nematodes capable of causing a deterioration of fine mycorrhizal rootlets such as has been demonstrated to occur as an early stage in the decline of western white pine known as pole blight (4). Many Heterodera larvae were found in soil samples collected from the Clarkia Peak, 4th of July Canyon, and Charlie Creek pole-blighted stands. They were found in the early spring and late fall and males were recovered from the Clarkia Peak samples during the latter part of October. Females (Fig. 1) were observed on white pine rootlets collected from the 4th of July Canyon diseased area also at the end of October. No Heterodera spp. were found in soil samples taken from healthy white pine stands.

Trichodorus elegans (1), was present in relatively high numbers in soil samples from all

---

[1]This paper is a portion of a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Idaho.

[2]Formerly Research Fellow, College of Forestry, University of Idaho, Moscow, Idaho. At present Research Assistant, Department of Plant Nematology, University of California, Davis, California.

FIGURE 1. A Heterodera sp. cyst embedded in a western white pine rootlet.

pole-blighted stands sampled. This nematode species was collected from soil under two apparently healthy stands of white pine south of the present range of the disease. Soil collected from beneath white pine trees previously killed by the disease did not yield specimens of T. elegans. However, soil samples collected at the edge of the diseased area from beneath living trees normally contained this stubby root nematode. White pine seedlings placed in a flat containing field soil infested with T. elegans maintained a large population of this nematode for at least 6 months. These observations indicate that western white pine is probably a host of this ectoparasite.

Specimens of nematodes in the genera Tylenchorhynchus, Criconema, and the species Criconemoides sphaerocephalum Taylor were found in soil from several diseased areas. These genera were not represented in soil samples taken from healthy pine stands. An undescribed species of Helicotylenchus was occasionally found in soil collected from both healthy and diseased stands.

Literature Cited

1. ALLEN, M. W. 1957. A review of the nematode genus Trichodorus with descriptions of ten new species. Nematologica 11: 32-62.
2. HOPPER, B. E. 1958. Plant-parasitic nematodes in the soils of southern forest nurseries. Plant Disease Reptr. 42: 308-314.
3. JACKSON, L. W. R. 1948. Deterioration of shortleaf pine roots caused by a parasitic nematode. Plant Disease Reptr. 32: 192.
4. LEAPHART, C. D., O. L. COPELAND, and D. P. GRAHAM. 1957. Pole blight of western white pine. U. S. Dept. of Agr. Forest Service, For. Pest Leaflet 16. 4 pp.
5. NICKLE, W. R. 1958. Nematodes associated with the rootlets of western white pine in northern Idaho. Master's Thesis, University of Idaho. 61 pp.

UNIVERSITY OF IDAHO, MOSCOW, IDAHO

SEED DETERIORATION AS A FACTOR IN NUB-ROOT PRODUCTION IN COTTON[1]

A. B. Wiles and J. T. Presley[2]

Summary

In the course of screening cotton seedlings for reaction to disease, numerous cotton seeds from diverse sources were germinated in sterile sand. Within certain lots of seed at least 50% produced seedlings which lacked primary radicles or had severely injured radicles. Such plants generally were delayed in emergence and tended initially to lack vigor. Healthy hypocotyls as well as healthy lateral roots emerged from the damaged radicles. When such seedlings were potted in sterile soil and grown to maturity in the greenhouse, however, they developed into plants which totally lacked true taproots. The planting of deteriorated seed is a major cause of this nub-root condition in mature cotton plants. The results obtained from naturally deteriorated seed were substantiated by using seed which were deteriorated under laboratory conditions.

INTRODUCTION

The condition in cotton plants aptly termed "nub-root" is essentially an abnormal abbreviation of the taproot. This injury may vary from severe where there is no taproot to moderate where only a portion of the taproot is lacking. In some cases a lateral root may partially take over the functions of the shortened taproot. Affected plants tend to wilt readily during periods of moisture stress and may topple over. In Mississippi mature nub-root plants have been found only in heavier soils where moisture stress is not critical until the middle of the season.

In the past nub-root has been attributed to such causes as strangulation of the roots by soil compaction and development of soil hardpan, and more recently by Stewart and Whitehead (3) to damage of roots of seedling plants by soil fungi. The writers, working separately, have found that the past history of the seed is important in the nub-root problem. This fact has apparently not been previously reported and should not be confused with breakdown of the radicles of cotton seedlings due to a nutritional deficiency reported by Presley and Leonard (2) and described further by Wiles (5) in 1959. In the latter case the primary radicle is injured after emergence from the seed, while in the nub-root condition the injury results from unfavorable environmental conditions imposed upon the seed.

RESULTS AND DISCUSSION

The possibility that nub-root could have early origins was observed in the course of evaluating cotton seedlings for their reaction to Verticillium wilt (4). In these studies acid-delinted cotton seed which had been treated with a standard fungicide were planted in steam-sterilized sand. Certain lots of seed produced a high percentage of abnormal seedlings (Fig. 1). Such seedlings were generally late in emergence and showed varying degrees of injury to the primary radicle. Healthy lateral roots were formed from the hypocotyl or from the remaining portion of the primary radicle.

A study was set up in the greenhouse to determine the course of development of such plants. One hundred young cotton seedlings with damaged primary radicles were transplanted to 8-inch clay pots, which contained steam-sterilized sandy loam soil. Each pot contained five of these plants and one plant with a normal primary radicle. After 6 weeks half the plants were lifted and the roots examined (Fig. 2); the remaining plants were allowed to remain potted for 3 months. In all cases where seedlings with injured radicles had been used the mature plants showed varying degrees of taproot injury or nub-root. The seedlings with healthy primary radicles grew into plants with normal taproots. It was thus shown that nub-root could occur where soil strangulation, hardpan, and fungi were not involved. The factors responsible for breakdown of the tissues of the primary radicle in the seed were enumerated in the recent

[1]Journal Paper (New Series) No. 815 of the Mississippi Agricultural Experiment Station; Cooperative Investigations of the Crop Research Division, Agricultural Research Service, United States Department of Agriculture, and the Mississippi Agricultural Experiment Station.

[2]Agent, United States Department of Agriculture, and Associate Plant Pathologist, Mississippi Agricultural Experiment Station, State College, Mississippi; and Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

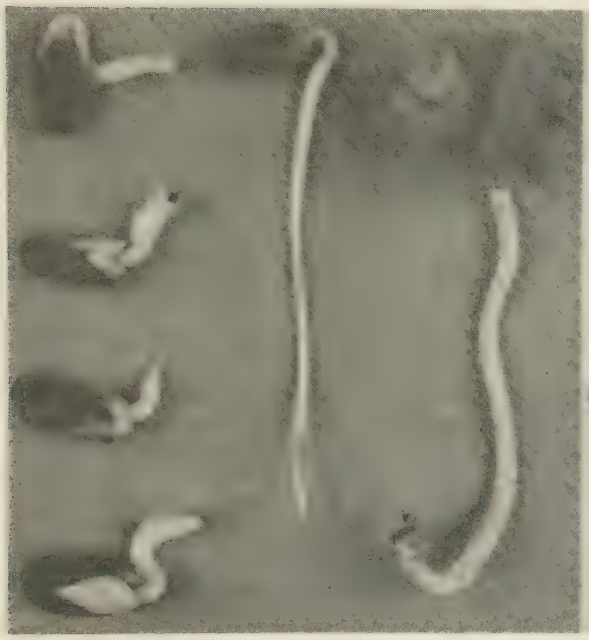


FIGURE 1. Young cotton seedlings showing at left, deterioration injury to primary radicle; at center, uninjured primary radicle; and, at right, lateral roots developing above injury.

FIGURE 2. Young cotton plants showing comparative plant size and root development. At left, five plants from deteriorated seed; and, at right, three plants from nondeteriorated seed. No taproot was present on the five at the left.

work of Presley (1). He found that time, temperature, and moisture profoundly affect seed viability and, further, that by manipulating these factors cotton seed could be artificially deteriorated. From seed deteriorated in this manner a high percentage of seedlings with damaged primary radicles was obtained.

It has thus been found that both naturally and artificially deteriorated cotton seed give rise to seedlings with damaged primary radicles, and mature plants may develop from such seedlings which show typical nub-root symptoms. These findings do not preclude the possibility that other factors may produce the same final damage. In the case of soil fungi, however, it is necessary to assume that the fungi destroy only a portion of the root and then, for some reason, the invasion is halted before the root is totally destroyed. Field and greenhouse observations by the writers have failed to substantiate this assumption. These results show the importance of using nondeteriorated seed as a means of improving stands in cotton. The dual effects of the past history of the planting seed and early seedling nutrition appear to warrant further investigation.

Literature Cited

1. PRESLEY, JOHN T. 1958. Relation of protoplast permeability to cotton seed viability and predisposition to seedling disease. Plant Disease Reptr. 42: 852.
2. PRESLEY, J. T., and O. A. LEONARD. 1948. The effect of calcium and other ions on the early development of the radicles of cotton seed. Plant Physiol. 23: 516-525.
3. STEWART, ROBERT B., and MARVIN D. WHITEHEAD. 1955. Nub-Root -- the expression of seedling disease in the mature cotton and flax plant. Phytopathology 45: 413-416.
4. WILES, ALFRED B. 1952. A seedling inoculation technique for testing cotton varieties for resistance to Verticillium wilt. (Abst.) Phytopathology 42: 288.
5. WILES, ALFRED B. 1959. Calcium deficiency in cotton seedlings. Plant Disease Reptr. 43: 365-367.

MISSISSIPPI AGRICULTURAL EXPERIMENT STATION AND CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

REACTION OF RHIZOCTONIA SOLANI ISOLATES TO CERTAIN CHEMICALS

J. B. Sinclair[1]

Abstract

Greenhouse and laboratory studies indicate that five different isolates of Rhizoctonia solani Kuehn differ statistically in their sensitivity to PCNB, captan and dichlone, alone or in combination, and that these differences may help explain, in part, the erratic performance of these chemicals when used in out-field tests for the control of cotton seedling damping-off.

INTRODUCTION

Certain chemicals, determined by greenhouse assay to be effective against damping-off of cotton seedlings (5, 6), incited by Rhizoctonia solani Kuehn, were placed in three out-field tests in 1957. Significant differences in stand count means were noted only in the field plot from which the culture of R. solani, used in the greenhouse tests, was originally isolated (8). Greenhouse and laboratory studies were made in 1957, 1958 and 1959 to test the degree of disease control against five isolates of the test organism by pentachloronitrobenzene (PCNB), N-trichloromethylmercapto-4-cyclohexene-1, 2-dicarboximide (captan), and 2, 3-dichloro-1, 4-naphthoquinone (dichlone), used alone or in combination[2]. The results from these tests are given. A portion of the data from greenhouse results were presented earlier in abstract form (7).

GREENHOUSE STUDIES

Greenhouse experiments were run using flats filled with steam-sterilized field soil infested with 10-day-old potato-sucrose broth cultures of the R. solani isolate. The flats were sowed approximately 2 weeks after inoculation with Deltapine 15 cotton seed, treated with Dow 9-B, in seven replicates with 50 seed per replicate. Inoculated-nontreated and noninoculated-nontreated flats served as controls (5, 6). All the isolates of the test fungus for both the greenhouse and laboratory studies were cultured from diseased cotton seedlings collected from different localities in Louisiana and one isolate received from Dr. Neil D. Fulton of Arkansas, labeled 118Q. The following code numbers were assigned to the isolates used: 22, from Tallulah; 34, from Waterproof; T (the isolate used in a greenhouse screening program (5, 6)), from Roosevelt; 4, from New Iberia; and A, for the Arkansas isolate. A preliminary greenhouse run showed isolates 22, 34, T and 4 to be representative of all the others collected in their reaction to the chemicals tested.

The chemical treatments used in the greenhouse experiments were PCNB plus captan and PCNB plus disodium ethylene bisdithiocarbamate (nabam) in equal proportions. These materials were mixed with 200 cc of water and applied as an "in-the-furrow" treatment (5,6) at a rate of 2 1/2 pounds per acre of formulated material for the wettable powders and 2 quarts per acre for nabam. Emergence and disease counts were taken at least 2 weeks after planting and treating and the data expressed as percentage of surviving healthy plants. Each experiment was repeated three times with essentially the same results. The data from two runs of each treatment and four isolates are given (Table 1).

Results from the greenhouse tests indicated that of the four isolates used, T, 4 and 22 were highly pathogenic and isolate 34 moderately pathogenic as shown by the means of surviving healthy seedlings in the inoculated-nontreated checks. The mean number of healthy seedlings for isolate 34 in runs 1 and 2 were 71 and 34 respectively. These means were significantly (.01 level) lower when compared with the means of their respective noninoculated-nontreated checks, but significantly (.01 level) higher when compared with the means of the inoculated-nontreated checks of the other three isolates. Where PCNB plus captan was used as a treatment, the means of surviving seedlings resulting from isolates 22, 4 and T were significantly (.01 level) lower when compared with means of the noninoculated-nontreated checks in both runs. T ranked fourth and was least affected by this treatment and the means from both runs were significantly (.01 level) below those of the other three isolates. Isolate 34 showed a mean significantly lower than that of the noninoculated-nontreated check at the .05 level in the first run and .01 level in the second run and was the most affected by PCNB and captan. Part of this can be attributed to the low pathogenicity of this isolate. Moderate control of damping-off by PCNB plus captan was

[1]Assistant Professor of Plant Pathology, Louisiana State University Agricultural Experiment Station, Baton Rouge, Louisiana.

[2]Coined names for chemicals of known composition (2, 9).

Table 1. Treatment means for percentage of surviving healthy cotton seedlings from 50 seed when grown in steam-sterilized field soil inoculated with the isolate of Rhizoctonia solani and treatment indicated.

Isolate	Treatment[a]					
	P plus C		P plus N		Check 1	
	1	2	1	2	1	2
34	85	54	74	65	71	34
22	59	14	9	53	1	0
4	7	13	61	9	0	6
T	1	9	42	27	0	0
Check 2	88	82	82	90	88	82
LSD .05	3	3	3	2	3	3
LSD .01	5	4	4	3	5	4

[a]P = PCNB, C = captan, N = nabam, 1, 2 = experiment number, Check 1 = inoculated-nontreated, Check 2 = noninoculated-nontreated.

noted where isolates 22 and 4 were used. Isolate 4 gave means significantly (.01 level) above that of T in both runs, but significantly (.01 level) below those of 22 and 34 except in the second run where there was no significant difference between the means of 4 and 22. When PCNB plus nabam was used as a treatment the means of surviving seedlings resulting from all isolates were significantly lower than the noninoculated-nontreated checks in both runs and the differences between each isolate were significant (.01 level). Isolate 34 was most affected by this treatment having a mean significantly (.01 level) above the means of the other three isolates in both runs. Isolate 22 was least affected in run 1 with a mean significantly below those of the other three, but in the second run, 4 was the least affected. The means from T showed this isolate to be second most affected by this chemical combination in both runs.

LABORATORY STUDIES

In this series of experiments isolates 22, 34 and T were used. Isolate A was substituted for 4. Sterile potato-sucrose agar measured in 200-cc portions was cooled to a point where it was still liquid. The chemicals to be tested were weighed and then mixed with the liquid agar. From each 200-cc portion, five Petri plates were poured and each resulting plate was considered a replicate. Each plate had two drops of 10% acetic acid added. Agar without any test chemical added served as a check. The chemicals tested were PCNB, captan, and dichlone, alone or in combination. The rates for these materials when used alone were 0.364 g of formulated material for 200 cc of agar. When used in combination the rates were 0.182 g of formulated material for 200 cc of agar for each chemical added. This was converted to a ppm of active ingredient basis (Table 2).

Table 2. Treatment means for amount of mycelial growth in cm for four isolates of R. solani listed having been grown on potato-sucrose agar mixed with chemicals indicated at the rate in ppm of active ingredient given.

Isolate	Treatment					
	PCNB (1370 ppm)		Captan (910 ppm)		Dichlone (910 ppm)	
	1[a]	2	1	2	1	2
34	0.0	1.1	0.0	0.0	1.8	0.0
22	2.2	2.3	0.8	1.3	8.3	5.4
A	1.6	1.9	1.9	1.9	8.1	4.7
T	1.9	2.0	0.8	1.3	8.1	4.7
LSD .05	0.2	0.1	0.4	0.1	0.3	0.6
LSD .01	0.3	0.2	0.5	0.1	0.5	0.9

[a]Experiment number.

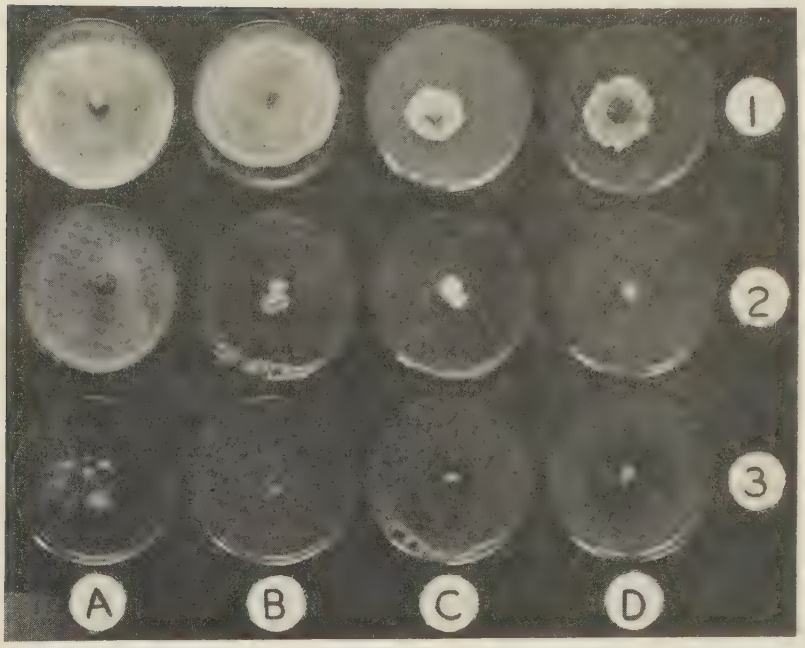


FIGURE 1. Growth of three isolates of Rhizoctonia solani on potato-sucrose agar mixed with either A) no chemical, B) dichlone, C) captan, or D) PCNB. 1 = isolate T, 2 = isolate 34, 3 = isolate 4.

All plates were seeded by taking bits of agar, less than 0.5-cm square, over which the mycelium of the test organism had grown, and placing one of them in the center of each plate. Diameter readings in cm of the resulting cultures were taken when check plates were completely covered by the mycelial growth or between 4 to 6 days after seeding (Fig. 1). The growth pattern was usually circular, but when the pattern was irregular, the measurement was taken between the widest points of growth. The experiments where the chemicals were used alone were repeated at least four times and those using the chemicals in combination were repeated at least twice.

Results from the laboratory experiments showed that growth of R. solani on potato-sucrose agar mixed with either PCNB, captan or dichlone differed significantly depending upon the isolate of the organism used (Table 2). Where combination chemical treatments were used little growth was noted in the case of captan plus dichlone, no growth resulted where PCNB plus captan was used, and PCNB plus dichlone exhibited an erratic pattern ranging from heavy to no growth.

Results in experiments where the chemicals were used alone, the least amount of growth in any run, regardless of treatment, was exhibited by 34. This isolate was slow growing as indicated by the check plates. The isolate least affected by PCNB was 22, followed by T and A, respectively. Isolates T, A, and 34 in decreasing order showed a growth rate significantly (.01 level) lower than that of 22. In the case of captan, A was least affected, followed by 22 and T, respectively. The differences between the means of A and the other three isolates were significantly (.01 level) lower. The results from experiments where dichlone was used alone were less consistent than the other two treatments. In both runs, 22 showed the most growth followed by A and T, respectively, but the differences were not significant. The growth of isolate 34 was significantly (.01 level) lower than that of 22 in both runs. In the second run, 22 again exhibited the most growth and A and T were significantly (.05 level) lower with identical means.

SUMMARY AND CONCLUSIONS

Results from in-the-furrow chemical treatments for field control of seedling damping-off in Louisiana have been inconsistent (8). Part of this lack of consistent performance might be attributed to differences in environmental conditions (3, 4), and the disease organisms involved (1). However, experimental data from field, greenhouse, and laboratory studies, presented here, show that there may be another factor to consider. Statistical differences between isolates of R. solani in their reaction to the test chemicals indicate that some of these isolates seem to be less affected than others by the same test chemical or combination of chem-

icals. Good chemical control of the disease might be obtained in a field test where a strain of R. solani similar to isolate 34 predominated. Isolate 34 was slow growing and not so pathogenic as the other isolates tested. Isolates 22, 4, A and T, are similar in that they grew more rapidly than 34 and were highly pathogenic. Yet highly significant differences in greenhouse and laboratory tests indicate that difference in field performance might be expected where these isolates or similar isolates might predominate. Isolate 22 grew better than did the other highly pathogenic isolates tested, not only on agar with PCNB and dichlone but also in two greenhouse tests. Isolate T was very pathogenic and generally least affected by any of the test chemicals.

The additional factor to consider when interpreting inconsistent field results from in-the-furrow experiments for chemical control of cotton seedling damping-off, where R. solani is involved as one of the disease organisms, is the relative effectiveness the test chemical or chemicals might have against the strain of this organism in the soil.

Literature Cited

1. FULTON, N. D., B. A. WADDLE, and R. O. THOMAS. 1956. Influence of planting dates on fungi isolated from diseased cotton seedlings. Plant Disease Reptr. 40: 556-558.
2. McCALLAN, S. E. A., LAWRENCE P. MILLER, and MARY A. MAGILL. 1955. Chemical names for active ingredients of fungicides. Phytopathology 45: 295-302.
3. RANNEY, C. D., and L. B. BIRD. 1956. Greenhouse evaluation of the in-the-furrow fungicides at two temperatures as a control measure for cotton seedling necrosis. Plant Disease Reptr. 40: 1032-1040.
4. RUSHDI, M., and W. F. JEFFERS. 1956. Effect of soil factors on efficiency of fungicides in controlling Rhizoctonia solani. Phytopathology 46: 88-90.
5. SINCLAIR, J. B. 1957. Laboratory and greenhouse screening of various fungicides for control of Rhizoctonia damping-off of cotton seedlings. Plant Disease Reptr. 41: 1045-1050.
6. SINCLAIR, J. B. 1958. Greenhouse screening of certain fungicides for control of Rhizoctonia damping-off of cotton seedlings. Plant Disease Reptr. 42: 1084-1088.
7. SINCLAIR, J. B. 1958. Reaction of four Rhizoctonia solani isolates to certain chemicals. (Abst.) Phytopathology 48: 398.
8. SINCLAIR, J. B., D. C. NEAL, E. K. CHANDLER, D. M. JOHNS, L. W. SLOANE, and D. WALTERS. 1958. Field screening of various fungicides for control of cotton seedling damping-off. Plant Disease Reptr. 42: 1372-1375.
9. ZENTMEYER, G. A. 1955. Coined names for fungicides. Phytopathology 45: 109.

LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE

NITROGEN SUPPLEMENT IN THE LOUISIANA-MISSISSIPPI RIVER DELTA AS A POSSIBLE CONTROL FOR VERTICILLIUM WILT OF COTTON

D. C. Neal[1] and J. B. Sinclair[2]

In 1958 the addition of extra nitrogen, 32 pounds of N per acre from nitrate of soda, the latter part of July proved beneficial in increasing the yield of Auburn 56 x Deltapine 14-312 in a Verticillium-wilt-infested area near Lake Providence, Louisiana. Notwithstanding heavy and uniform wilt infestation over the entire experimental plot, the yield in October was estimated to range between 1 1/2 to 1 3/4 bales per acre.

In 1959 five standard commercial varieties, two new strains and Auburn 56 x Deltapine 14-312 were included in a test in which extra nitrogen, the same source and amount of N per acre as in 1958, was applied on July 24. The test was planted on May 12. All of the cottons in the test were mature and ready for harvest the latter part of September.

A reduction in plants with aboveground symptoms of Verticillium and those killed by the disease was apparent in six of the eight entries listed when the plot was examined on October 7.

The yields are given in Table 1. Highest yields of lint per acre in the order named were from Strain 13, La. Hybd. 3314, Dixie King and Deltapine 15 (a tie), Deltapine Smooth Leaf,

Table 1. Yield of commercial varieties and new strains of cotton on Verticillium wilt infested soil following application of supplemental nitrogen in July 1959 at Lake Providence, Louisiana[a].

Variety or strain	Pounds of lint per 253-foot row section, Replication 1	2	3	4	Mean	Pounds of lint per acre	% lint
Strain 13	22.7	21.1	18.6	27.6	22.5	1108.3	39.2
La. Hybd. 3314	19.5	20.5	27.3	18.0	21.3	1049.2	38.8
Deltapine 15	20.6	19.9	21.8	20.6	20.7	1019.7	39.8
Dixie King	22.0	19.9	19.5	21.4	20.7	1019.7	36.6
Deltapine Smooth Leaf	26.3	17.7	21.6	16.0	20.4	1004.9	37.7
Auburn 56 x Dp. 14-312	19.0	19.6	20.2	19.6	19.6	965.5	39.3
Stoneville 7	19.1	21.8	17.6	17.5	19.0	935.9	38.2
Stardel	11.5	21.0	18.6	19.5	17.7	871.9	38.7
L.S.D. .05					ns	ns	
L.S.D. .01					ns	ns	

[a]Date of harvest November 19 and 20.

Auburn 56 x Deltapine 14-312, Stoneville 7, and Stardel. Yield differences were not statistically significant. Three of the commercial varieties and the two new strains yielded in excess of 2 bales (500 lb. min.) per acre, and in the remaining three entries the yields were approximately 1.9, 1.8 and 1.7 bales per acre, respectively.

The results obtained to date in these preliminary field tests indicate that extra N applied late in July in the amount indicated may be important in increasing cotton yields in certain areas infested with Verticillium wilt.

LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE

[1]Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

[2]Assistant Professor of Plant Pathology, Louisiana Agricultural Experiment Station.

EFFECTS OF SOIL FUMIGANTS ON THE OCCURRENCE OF NEMATODES IN FIELD BINS[1]

Earl B. Minton[2], Eldon J. Cairns[3], and Albert L. Smith[2]

Abstract

From 1957 to 1959 tests were conducted to determine the effectiveness of soil fumigants for eradication of nematodes in 20 field bins located on an Independence (Cahaba) loamy fine sand near Tallassee, Alabama. In November 1957 four liquid nematocides injected 6 inches deep, MC-2 used alone, and combinations of liquids with MC-2[4] did not eradicate nematodes to a depth of 31 inches. Blanket treatment of the bins in March 1958 with a mixture of Nemagon, D-D, and Dowfume W-85 injected 6 and 18 inches deep followed by two applications of MC-2, 11 and 28 days later reduced nematode population to a low level (15 nematodes in 628 soil samples). Two nematodes were found in the 0- to 10-inch sampling depth and 13 in the 10- to 20-inch depth. The same bins were retreated on June 10, 1958 with Telone injected 12 inches deep and with MC-2, ten days later. The bins remained free of stylet-nematode contaminants for 12 months. A few Trichodorus contaminants were found in 14 bins 12 and/or 18 months after treatment. The data indicate that field bins can be freed of stylet nematodes by intermittent use of liquid fumigants injected at 6-, 12-, and 18-inch depths followed by applications of MC-2. Intensive sampling was necessary to detect low numbers of survivors.

INTRODUCTION

The effect of soil fumigation on populations of plant-parasitic nematodes has been studied by previous investigators (3, 4, 6). Yields of various crops were increased by soil fumigation, even though the nematodes were not eradicated and populations were sometimes back at a high level after a short time. The rate of nematode increase after fumigation varied for different species.

Frazier and Dennett (2) sterilized with chloropicrin small outdoor beds used to determine the effect of root-knot nematodes on tomatoes. Previous workers have not studied the effect of single species of plant-parasitic nematodes on cotton under field conditions. Tests were initiated in 1957 to eradicate soil-borne nematodes in field bins and to determine the rate of recontamination while studying the pathogenicity of certain individual species of plant-parasitic nematodes on cotton. The latter phase of this investigation will be presented elsewhere (5).

MATERIALS AND METHODS

Open bottom field bins were constructed on Independence (Cahaba) loamy fine sand at the Plant Breeding Unit near Tallassee, Alabama. The bins were 20 by 24 feet and were separated by 15 feet. Walls were of concrete blocks, which extended approximately 16 inches below the soil surface and 8 inches above. The outside surfaces of the walls were painted with a waterproofing compound.

Precautionary measures were taken to prevent contamination. The area was fenced with 1-inch mesh wire, rubber boots worn by the workers and all tools were washed in a 10% formalin solution, nematocides and herbacides were applied in the alleys in 1958 and 1959, and river water used for supplemental irrigation in 1959 was treated with a nematocide.

A series of three consecutive tests for eradication of nematodes with various combinations of materials were conducted during the investigations. Fumigants used were Dowfume W-85 (83% 1,2-dibromoethane), methyl bromide = MC-2 (98% bromomethane, 2% trichloronitromethane), Telone (1,3-dichloropropene), Dorlone (75.2% 1,3-dichloropropene, 18.7% 1,2-dibromoethane), D-D (50%

---

[1]Cooperative investigations, Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the Departments of Agronomy and Soils and of Botany and Plant Pathology, of the Auburn University Agricultural Experiment Station, Auburn, Alabama.

[2]Respectively, Plant Physiologist and Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

[3]Nematologist, Auburn University.

[4]Mention of specific instruments or trade names is made for identification and does not imply any endorsement by the United States Department of Agriculture.

1,3-dichloropropene, 50% 1,2-dichloropropane), and DBCP = Nemagon (97% 1,2-dibromo-3-chloropropane). Liquid fumigants were applied with a McLean Fumigun with a 6-, 12-, or 18-inch injection point on 12-inch centers and MC-2 was applied under polyethylene tarpaulins sealed at the soil surface outside the bins.

Nematode survival or recontamination was determined by examination of soil samples. Each sample consisted of 8 to 10 borings collected with a soil sampling tube. In 1957 nematodes were separated from the soil by the modified Baermann funnel technique used by Christie and Perry (1) and in 1958-59 by the Seinhorst elutriator (7).

RESULTS AND DISCUSSION

Test 1: Twelve bins were treated with Dowfume W-85 at the rate of 10 gallons per acre injected 6 inches deep and MC-2 at 3 pounds per 100 square feet on May 28, 1957. The plastic cover was left in place 24 hours. A week later, prior to planting cotton, duplicate bins were seeded with single-species cultures of root-knot nematodes. Soil samples were collected from each bin 5, 12, and 22 weeks after fumigation. Non-stylet nematodes were found in all bins at every sampling date. Low populations of Trichodorus, Tylenchorhynchus, and Aphelenchoides contaminants were found in the control and seeded bins at the 22-week sampling date. Contaminants were more abundant near the edge of the bins and probably entered from the outside or survived treatment better near the edges. In 1957 large numbers of several species of nematodes were associated with the grass sod maintained in the alleys. Therefore, nematocides and herbicides were used to control nematodes and the grass in the alleys in subsequent tests. Contaminant nematodes could have occurred in the untreated river water used twice for supplemental irrigation during 1957.

During fumigation, approximately 1250 pounds per acre of bromine was applied. In July and August, when the available soil moisture was low, bromine toxicity was observed on the terminal leaves and stems of cotton. The symptoms disappeared when the available soil moisture was increased by irrigation or rain. Therefore, lower rates of MC-2 and Dowfume W-85 were used in subsequent tests.

Test 2: During October and November 1957, the liquid and gaseous nematocides listed in Table 1 were applied to single bins which had not previously been fumigated. MC-2 was applied at the rate of 1 1/2 pounds per 100 square feet and Dowfume W-85, Nemagon, D-D, and Dorlone were applied at the rates of 8, 2, 32, and 24 gallons per acre, respectively. The liquids were injected 6 inches deep. Then the bins were covered with plastic sheets, and MC-2 was applied. The cover was removed 10 days later, and the first series of soil samples was collected at that time. Additional samples were collected 70 and 140 days after fumigation. All bins were fallowed during this test. Dowfume W-85, Nemagon, D-D, Dorlone, and MC-2 alone and combinations of the first three liquid nematocides with MC-2 did not eradicate nematodes in the surface 8 inches of soil, even though the bins were covered for 10 days after treatment. Liquid (Dowfume W-85 and D-D) and gaseous (MC-2) nematocides appeared to be as effective when used separately as when the two types of materials were applied in combination. Nemagon + MC-2 was superior to Nemagon alone.

Test 3: In view of the difficulty in eradicating nematodes in tests 1 and 2, it was decided that the depth of nematode distribution should be determined before further treatments. Nematodes may have survived either within the treated zone or at depths below chemical injections. Therefore, soil samples were collected from two bins and one alley on March 5 and 27, 1958, to determine the distribution of nematodes at depths indicated in Table 2. In May 1957 one bin was fumigated as indicated in test 1 and then seeded with a culture of M. incognita acrita prior to planting cotton. In November 1957 the other bin was fumigated with Nemagon, 2 gallons per acre, and clean-fallowed. The alleys were untreated and served for comparison. Nematode populations were essentially the same in the two bins at both sampling dates and similar populations occurred in the alleys for both dates. The average numbers of nematodes per pint of soil are presented in Table 2. In general, nematodes were more abundant at all depths examined in the non-fumigated alleys. The population level decreased with depth, but plant-parasitic nematodes were found at the maximum depth sampled.

In previous tests liquid fumigants, injected 6 inches deep, and MC-2 used alone and various combinations of the two types of nematocides did not eradicate nematodes in the top 31 inches of soil. Therefore, blanket treatments as indicated herein were applied to all bins in 1958. During June 1957 12 bins were fumigated as indicated in test 1; then 10 of the bins were

Table 1. Effect of nematocides on soil-inhabiting nematodes (number per pint of soil from surface 8 inches) at 10, 70, and 140 days after treatment, 1957-58.

Time of examination and nematocide	Meloidogyne	Criconemoides	Trichodorus	Pratylenchus	Xiphinema	Spiral	Saprophagous
Before fumigation:							
MC-2	5	35	5	10	0	0	1860
MC-2 + Dowfume W-85	0	14	500	70	190	25	2901
Dowfume W-85	380	10	750	50	70	80	1748
MC-2 + Nemagon	0	5	425	110	25	15	1920
Nemagon	150	0	290	50	70	100	1409
MC-2 + D-D	0	5	470	70	25	5	2325
Dorlone	20	0	130	80	0	0	1940
D-D	100	0	600	30	41	80	1428
10 days after fumigation:							
MC-2	0	0	0	0	0	0	3
MC-2 + Dowfume W-85	0	0	0	0	0	0	19
Dowfume W-85	0	0	0	0	0	0	56
MC-2 + Nemagon	0	0	1	0	0	0	6
Nemagon	0	20	30	10	0	0	2156
MC-2 + D-D	0	0	0	3	0	0	11
Dorlone	0	0	0	0	0	4	0
D-D	0	0	0	0	0	0	315
70 days after fumigation:							
MC-2	0	0	0	0	0	0	1
MC-2 + Dowfume W-85	0	0	0	0	0	0	5
Dowfume W-85	0	0	0	2	0	0	14
MC-2 + Nemagon	0	0	0	0	0	0	9240
Nemagon	0	0	11	23	0	0	1436
MC-2 + D-D	0	0	3	1	0	0	131
Dorlone	0	0	0	0	0	0	0
D-D	0	0	0	0	0	0	3
140 days after fumigation:							
MC-2	0	0	0	0	0	0	90
MC-2 + Dowfume W-85	0	1	0	0	2	0	260
Dowfume W-85	0	0	0	0	3	0	260
MC-2 + Nemagon	0	0	0	0	1	0	133
Nemagon	0	55	10	0	268	0	1831
MC-2 + D-D	0	0	0	0	4	0	583
Dorlone	0	0	0	0	1	0	37
D-D	0	0	0	0	0	0	28

Table 2. Distribution of nematodes (average number per pint of soil) by soil depths in field bins and alleys in March 1958.

Place sampled and soil depth (inches)		Saprophagous	Meloidogyne	Pratylenchus	Criconemoides	Trichodorus	Xiphinema	Dorylaimus	Spiral
Field bins:	0-1/2	738	0	0	0	22	0	5	0
	1-3	702	7	5	1	31	3	1	13
	5-7	195	5	3	2	45	1	0	1
	11-13	61	1	1	0	10	0	0	0
	17-19	6	1	0	0	7	1	1	0
	23-25	0	0	0	0	1	1	4	0
	29-31	1	0	0	0	0	0	1	0
Alleys:	0-1/2	6069	0	30	4	24	0	268	0
	1-3	2590	10	105	31	102	15	374	0
	5-7	808	10	17	15	170	35	150	0
	11-13	37	0	4	5	29	8	26	0
	17-19	20	2	12	2	18	10	10	0
	23-25	36	0	3	0	9	1	13	0
	29-31	17	0	0	1	4	0	8	0

Table 3. Effect of Nemagon, Dowfume W-85, and D-D mixture followed by two applications of MC-2 on soil-inhabiting nematodes (per pint of soil) at different depths and periods after treatment, 1958.

Days after treatment	Sampling depth (inches)	Bins sampled	Samples per bin	Stylet	Non-Stylet
2	0-10	1-20	1	0	0
7	10-20	1-20	1	2 (Bin 2)	5 (Bins 2, 3)
12	0-10	1-20	3	0	2 (Bins 2, 3)
	10-20	1-20	3	1 (Bin 1)	1 (Bin 2)
19	0-10	1,3,8,17[a]	12	0	0
	10-20	1,3,8,17[a]	12	0	1 (Bin 8, plot 7)
	20-30	1,3,8,17[a]	12	0	0
25	0-10	Plot 5 and 7[b]	72	0	0
	10-20	of bin 8	72	1 (Plot 7)	2 (Plots 5, 7)
	20-30	do.	72	0	0
39	0-10	Plot 10 of[b]	36	0	0
	10-20	bin 8	36	0	0
	20-30	do.	36		

[a]Each bin was divided into 6 equal-sized plots.
[b]Each plot was divided into 36 equal-sized subplots.

Table 4. Trichodorus contaminants per pint of soil (surface 8 inches) per bin at two distances within bins 12 and 18 months after fumigation, 1959.

Time of examination and nematodes seeded in bin	0-3 feet	3-10 feet
12 months:		
None (control)	87	0
None (control)	7	0
None (control)	3	0
Meloidogyne incognita acrita	6	0
Meloidogyne incognita	5	0
Rotylenchus brachyurus	4	10
Rotylenchus brachyurus	18	0
Helicotylenchus nannus	74	0
Hoplolaimus tylenchiformis	150	0
Rotylenchulus reniformis	20	0
18 months:		
None (control)	3	0
None (control)	34	17
None (control)	54	24
None (control)	1	3
None (control)	93	7
None (control)	13	5
Meloidogyne incognita acrita	19	6
Meloidogyne incognita	27	3
Rotylenchus brachyurus	0	1
Rotylenchus brachyurus	20	0
Helicotylenchus nannus	24	2
Hoplolaimus tylenchiformis	19	3

seeded with single-species cultures of root-knot nematodes and two bins were not reinfested. One or more species of contaminant plant-parasitic nematodes occurred in these bins 5 1/2 months after fumigation. Stylet nematodes were not eradicated in all bins treated in test 2. A mixture of Nemagon, Dowfume W-85, and D-D at the rates of 2 quarts, 2 and 7 gallons per acre, respectively, was injected 6 and 18 inches deep on March 30. Two applications of MC-2 at 1 pound per 100 square feet were made 11 and 28 days later. After these three treatments soil samples were collected at three depths and at the time intervals shown in Table 3.

Nematodes were not found in the samples collected from the top 10 inches of soil 2 days after treatment (Table 3). Eleven nematodes were found in 140 samples examined during the next 10 days. Four nematodes were found in 468 samples examined during the next 27 days. These nematodes occurred in a fourth bin. Two of the survivors were in the 0- to 10-inch sampling depth and 13 in the 10- to 20-inch depth. Intensive sampling of the selected bins indicated effective killing occurred at the edges and in the corners as well as in the center of the bins. The treatments gave almost complete eradication, and intensive sampling was required to detect the rare survivors.

In test 3, 13 of the survivors were found in the 10- to 20-inch sampling depth. Telone at the rate of 22 gallons per acre was injected 12 inches deep on June 10 and MC-2 was applied at the rate of 1 pound per 100 square feet on June 28 to eradicate the few survivors. Twelve bins were seeded with a desired culture of plant-parasitic nematodes on July 25; cotton, tomato, and soybean were then planted intermingled in rows in all bins. Vetch was planted in September 1958, and cotton was again planted in April 1959.

Soil samples were collected from each bin at frequent intervals to determine the rate of contamination. Non-stylet nematodes were found in all bins at every sampling date. Small numbers of Trichodorus occurred in 10 and 12 bins at the 12- and 18-month sampling dates, respectively (Table 4). Contaminants were as abundant in the control bins as in those seeded with nematodes. Apparently contaminants occurred earlier near the edge of the bins and gradually spread inward. Trichodorus was the only species of plant-parasitic nematode found in the alleys during 1958 and 1959. It could have been carried into the bins by small rodents, rain-splashed or wind-blown soil.

A series of five consecutive treatments, two liquid and three gaseous, applied at intervals of 10 days or more were required to eradicate nematodes in test 3.

Literature Cited

1. CHRISTIE, J. R., and V. G. PERRY. 1951. Recovering nematodes from soil. Proc. Helminthol. Soc. Wash. D.C. 18: 106-108.
2. FRAZIER, W. A., and R. K. DENNETT. 1949. Isolation of Lycopersicon esculentum type tomato lines essentially homozygous to root knot. Proc. Amer. Soc. Hort. Sci. 54: 225-236.
3. GOOD, J. M., and A. E. STEEL. 1958. Control of sting nematodes for two growing seasons by soil fumigation. Plant Disease Reptr. 42: 1364-1367.
4. HOLLIS, JOHN P., and MAX J. FIELDING. 1958. Population behavior of plant parasitic nematodes in soil fumigation experiments. Louisiana Agr. Exp. Sta. Bull. 515. 23 pp.
5. MINTON, EARL B., ALBERT L. SMITH, and ELDON J. CAIRNS. 1960. Population build-up and pathogenicity of reniform, root-knot, lance, and spiral nematodes on cotton, soybean, and tomato in field bins. (Abst.) Phytopathology. (In press)
6. PERRY, V. G. 1953. Return of nematodes following fumigation of Florida soils. Proc. Florida State Hort. Soc. 66: 112-114.
7. SEINHORST, J. W. 1956. The quantitative extraction of nematodes from soil. Nematologica. 1: 249-267.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE AND AUBURN UNIVERSITY AGRICULTURAL EXPERIMENT STATION, AUBURN, ALABAMA

PRELIMINARY STUDIES WITH DBCP COTTON SEED TREATMENT FOR CONTROLLING THE ROOT-KNOT NEMATODE[1]

John H. O'Bannon and Harold W. Reynolds[2,3]

Summary

Experiments were conducted in greenhouse pot culture and in ground bed plots to investigate the treatment of cotton seed with DBCP for the control of the cotton root-knot nematode. The phytotoxic effects of the chemical on seed germination and seedling growth also were studied. Very good root-knot nematode control was obtained at rates of 4.30, 2.15, and 1.70 pounds of technical DBCP per 25 pounds of seed, the amount generally used to plant an acre. No phytotoxic effects of any dosage rate at mean air temperatures of 18° to 25° C were encountered. When the mean air temperature was 32.7°, seed germination was adversely affected.

Attack by the cotton root-knot nematode, Meloidogyne incognita acrita Chitwood, decreases the chances of cotton seedlings developing normally, especially in sandy soils. Heavy attack of young seedlings reduces vigor and contributes to damping-off.

Protection of cotton seedlings by the use of chemical seed treatment for the control of various organisms is a well-established practice. Controlling nematodes with soil fumigants is also a common practice. Seed treatment for the control of nematodes, however, has not been practical because most nematocides are phytotoxic. An exception under certain conditions, so far as cotton is concerned, is DBCP[4]. Studies were initiated on the feasibility of applying it directly to cotton seed for root-knot nematode control.

MATERIALS AND METHODS

Acid-delinted cotton seed (Acala 44) was treated with technical DBCP at various rates. Preparation consisted of blending three parts by weight of technical DBCP with one part by weight of Micro-Cel[5] in an electric mixer. Micro-Cel at this ratio is in the range of its adsorptive capacity. Enough cotton seed for the desired seeding rate per acre equivalent was treated with a sticker[6]. The amount of sticker used varied from 4 to 8% by weight depending upon the dosage rate of DBCP desired. After application of the sticker to the seed, the DBCP - Micro-Cel mixture of the appropriate dosage was applied to the seed and the seed shaken to obtain uniform coverage. After treatment the seed was stored in screw-top glass jars to prevent any loss of chemical through volatilization.

GREENHOUSE POT EXPERIMENTS

Two experiments were conducted under greenhouse conditions. In the first, Acala 44 cotton seed was treated with technical DBCP at rates of 2.15, 1.70, and 0.85 pounds per 25 pounds of seed.

Forty-eight 6-inch pots filled with sandy loam free of root-knot nematodes were embedded in sand in the greenhouse bench. Cotton root-knot nematode eggs were hatched as described by Godfrey (1). Fifteen hundred viable larvae in water were added to each of 24 pots. The remaining 24 pots were left uninoculated for germination and growth studies. The experiment was randomized in blocks and replicated six times. Five seeds were planted in each pot. This seeding rate was estimated as equivalent to a field rate of 20 to 25 pounds per acre.

The plants were grown for 40 days at a mean air temperature of 24.6° C. Then they were harvested and the roots carefully washed to remove excess soil. Plant survival and growth measurements were taken. Each plant was given a relative root-knot index rating as described by Smith and Taylor (2).

---

[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Arizona Agricultural Experiment Station.

[2] Nematologists, Crops Research Division, Cotton Research Center, Tempe, Arizona.

[3] The writers wish to acknowledge that Dr. J. E. Chilton, plant pathologist, Arizona Fertilizer and Chemical Company Inc., assisted in seed preparation.

[4] A product furnished by the Shell Chemical Company under the trade name Nemagon Soil Fumigant. Active ingredient: 1,2-dibromo-3-chloropropane, 17.3 pounds by weight per gallon.

[5] A synthetic calcium silicate, product of the Johns-Manville Corp.

[6] A methyl cellulose derivate manufactured by the Cortex Chemical Co., Phoenix, Arizona.

Table 1. Mean relative root-knot index and percentage survival of DBCP seed treatments in root-knot nematode-infested soil, six replications.

Treatment	Rate per acre (pounds)	Relative root-knot index[a]	% survival[b]
DBCP	2.15	0.10	96.6
DBCP	1.70	0.43	93.3
DBCP	0.85	1.43	83.3
Control (untreated)		2.72	93.3
L.S.D. .05		0.3	
L.S.D. .01		0.4	

[a]Relative root-knot index: 0 = no infection, 1 = slight infection, 2 = light infection, 3 = moderate infection, 4 = heavy infection.
[b]Percentage survival based on five seeds per pot.

Table 2. Mean growth of seedlings from DBCP treated and untreated seed in root-knot-infested and non-infested soils, six replications.

Treatment	Rate per acre (pounds)	Height (in cm)	
		Soil inoculated with root-knot nematodes	Soil free of root-knot nematodes
DBCP	2.15	13.54	13.11
DBCP	1.70	13.54	12.85
DBCP	0.85	13.79	12.35
Control (untreated)		12.24	11.81
L.S.D. .05		1.0	

The relative root-knot index in Table 1 shows that the two higher rates gave very good root-knot control. A highly significant difference was also obtained at the 0.85 pound rate. Differences in plant survival were not significant.

Table 2 shows no phytotoxicity to seedlings as a result of the seed treatment.

In the second experiment, Acala 44 cotton seed was treated with technical DBCP at rates of 4.30 and 2.15 pounds per 25 pounds of seed as in the previous experiment.

Thirty-six 6-inch pots were prepared as before. Half of the pots were inoculated with 1200 root-knot larvae; the remaining pots (free of nematodes) were used in phytotoxicity studies. The experiment was randomized in blocks and replicated six times. Five seeds were planted in each pot and grown for 33 days at a mean air temperature of 31.1° C. The plants were harvested and plant survival and growth measurements were recorded. The roots were given a relative root-knot rating.

Excellent root-knot control was obtained with 4.30 and 2.15 pounds, as indicated by the relative root-knot index shown in Table 3. Plant survival, however, was less with DBCP-treated than with untreated seed. This indicated adverse effects of the chemical on seed at higher temperatures.

Growth measurements at harvest (Table 4) show no significant differences between treated and untreated seed in either nematode-infested or non-infested soil. Surviving seedlings grew normally.

GROUND-BED EXPERIMENTS

Two experiments were conducted in ground-bed plots to study the effects of temperature on germination of treated seed under simulated field conditions. Acala 44 cotton seed was treated with technical DBCP at rates of 4.30 and 2.15 pounds per 25 pounds of seed prepared as before.

Sixty seeds spaced 1 1/2 inches apart were planted in rows. The 1 1/2-inch spacing was used as it most nearly approached field spacing at the 20 to 25 pound per acre planting rate.

The experiments, consisting of two treated rows and an untreated row, were randomized and replicated five times.

In the first experiment seedlings were grown for 41 days at mean air temperature of 32.7° C. In the second experiment seedlings were grown for 35 days at a mean air temperature of 21.5°. Survival counts were made as shown in Table 5.

Table 3. Mean relative root-knot index and percentage survival of DBCP seed treatments in root-knot nematode-infested soil, six replications.

Treatment	Rate per acre (pounds)	Relative root-knot index[a]	% survival[b]
DBCP	4.30	0.03	66.6
DBCP	2.15	0.16	56.6
Control (untreated)		3.63	90.0
L.S.D. .05		0.2	32.6
L.S.D. .01		0.3	

[a]Relative root-knot index: 0 = no infection, 1 = slight infection, 2 = light infection, 3 = moderate infection, 4 = heavy infection.
[b]Percentage survival based on five seeds per pot.

Table 4. Mean growth of seedlings from DBCP treated and untreated seed in root-knot infested and non-infested soils, six replications.

Treatment	Rate per acre (pounds)	Height (in cm)	
		Soil inoculated with root-knot nematodes	Soil free of root-knot nematodes
DBCP	4.30	16.00	15.62
DBCP	2.15	15.67	13.77
Control (untreated)		15.82	15.19
L.S.D.		NS	NS

Table 5. Mean survival of DBCP treated and untreated cotton seed at two temperatures, five replications.

Treatment	Rate per acre (pounds)	% survival	
		32.7°C	21.5°C
DBCP	4.30	24.0	61.0
DBCP	2.15	30.0	56.0
Control (untreated)		46.0	58.0
L.S.D. .05		13.4	NS

In the first ground-bed experiment, conducted at a mean air temperature of 32.7° C, percentage survival of the treated seed at both rates was significantly reduced. In the second ground-bed experiment, conducted at a mean air temperature of 21.5°, no significant difference between any treatment and the control was noted, again indicating that chemical toxicity occurs at higher temperatures.

DISCUSSION

These preliminary studies have shown that nematode control is obtained by seed treatment with DBCP. No effects on germination or growth were encountered at mean temperatures of 18° to 25° C during cotton-planting season. When temperatures were above 31° germination was adversely affected.

Applying a nematocide to the seed eliminates the use of extra equipment and time involved in preplanting application. Placing the nematocide on the seed has the advantage of utilizing it where and when it is most needed, since it gives protection to the developing seedlings by controlling nematodes in the soil immediately around the developing root.

This method of control cannot be recommended for use until it has been tested extensively on a field scale.

Literature Cited

1. GODFREY, G. H. 1931. Some techniques used in the study of the root-knot nematode, Heterodera radicicola. Phytopathology 21: 323-329.
2. SMITH, A. L., and A. L. TAYLOR. 1947. Field method of testing for root-knot infestation. Phytopathology 37: 85-93.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE AND OREGON AGRICULTURAL EXPERIMENT STATION

ADDITIONAL CHARACTERISTICS OF PANICUM MOSAIC VIRUS[1]

W. H. Sill, Jr. and M. K. Desai[2]

Abstract

The Panicum mosaic virus does not appear to be seedborne. It may be stored successfully as desiccated tissue in a refrigerator for at least 2 years. Setaria lutescens is a new systemic host.

Panicum mosaic, a virus disease of switchgrass (Panicum virgatum), was described in 1957 (1, 2). Since that time it has been seen each year in the same perennial clones. Some of the more susceptible of these have died out but most persist and the majority are not stunted badly even though leaf mottling and mosaic symptoms are clear during the rapid growth period of early summer.

This virus can be spread easily in the greenhouse by mechanical abrasive inoculation techniques, but there is no clear evidence yet that it spreads in this way naturally in the field. Also there is little, if any, observational evidence of spread by insects or mites.

This study was initiated primarily to determine whether the virus might be seedborne. Seed was collected from infected Digitaria sanguinalis and P. virgatum plants (including the following selection numbers: 6248, 6184, 2275-8, 2144-12, 2278-9, and Nebraska 28). After harvest the seed was stored 6 months or more in a cold room before planting. Both species were planted separately in 8-inch pots during the winter in a greenhouse maintained at 70° F. Plants were examined weekly after emergence for the development of virus-like symptoms. In all, 280 D. sanguinalis and 564 P. virgatum plants were observed. None of the former and only 14 of the latter showed possible virus-like symptoms of any kind. These 14 plants were used as an inoculum source and abrasive inoculations were made from each diseased plant to 10 healthy P. virgatum and Setaria italica plants. None of the inoculated plants developed symptoms during 2 months of observation. Seed transmission apparently did not occur in either P. virgatum or D. sanguinalis.

As a control, 108 healthy P. virgatum and 79 D. sanguinalis plants were grown from seed collected from healthy plants. None developed virus-like symptoms of any type during a 2-month observational period.

Several plant species were inoculated by routine carborundum abrasive techniques with extracts obtained from diseased P. virgatum leaves which had been desiccated over $CaCl_2$ and stored at 2° to 4° C. Tissue stored 2 years was still infectious in 10/107[3] P. virgatum, 5/38 S. italica, 3/27 Panicum miliaceum, and 4/19 Setaria lutescens plants.

S. lutescens is a new host of the virus. It develops yellow mosaic-mottling symptoms and some stunting. Two plant species inoculated, Setaria viridis (34 plants) and Digitaria eriantha (107 plants), failed to develop symptoms and probably are immune.

Literature Cited

1. SILL, W. H., Jr. 1957. Panicum mosaic, a new virus disease of Panicum virgatum and related grasses. (Abst.) Phytopathology 47: 31.
2. SILL, W. H., Jr., and R. C. PICKETT. 1957. A new virus disease of switchgrass, Panicum virgatum L. Plant Disease Reptr. 41: 241-249.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, KANSAS STATE UNIVERSITY, MANHATTAN, KANSAS

[1]Contribution No. 567, Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station, Manhattan. Botany serial No. 728.
[2]Associate Plant Pathologist, Kansas Agricultural Experiment Station, Manhattan, and Principal, Agricultural College, Junagadha, Gujrath State, India, I. C. A. -India-KSU visitor.
[3]Numerator = plants infected. Denominator = plants inoculated.

OBSERVATIONS ON THE NATURAL SPREAD OF THE SO-CALLED PEACH STUNT VIRUS IN A CALIFORNIA PEACH ORCHARD[1]

H. Keith Wagnon, James R. Breece, Harold E. Williams and Dan Y. Rosenberg[2]

INTRODUCTION

In May 1952, while peach budwood source trees in an orchard in San Joaquin County, California were being inspected, disease symptoms consisting of shortened internodes and rosetted foliage and regarded to be the result of "peach stunt" virus infection were encountered in 17 peach trees. All infected trees were of the Paloro variety and were confined to one of four adjacent varietal blocks making up a 13-year-old planting of 14 acres. In addition to the block of Paloro trees, the planting consisted of blocks of Johnson, Libbee and Gaume varieties (Fig. 1).

Occasional visits to the Paloro block were made during 1953-1958 for the purpose of demonstrating the disease symptoms to other personnel. Although no complete survey of the planting was made during these occasional visits, it was apparent that the incidence of the disease was increasing in the Paloro block. A survey of the entire planting made in June 1959 showed considerable spread of the disease within the Paloro block and extension of it into the other three varietal blocks[3].

IDENTITY OF THE DISEASE

The virus nature of the disease was demonstrated when leaf symptoms developed on two of three young Peak peach test plot trees 1 year after being graft-inoculated with budwood from one of the affected Paloro orchard trees. Considerable confusion exists as to the proper name for this disease. In California it has been called peach stunt, and has been regarded to be the same as "'Stunt' of Peach" reported in Washington by Reeves (12) in 1951. Reeves pointed out that the symptoms of "stunt" were similar to those reported for the rosette mosaic, sour cherry yellows and prune dwarf virus diseases in peach. Muir peach dwarf should also be listed as having similar symptoms, but G. L. Stout and L. C. Cochran, who participated in the original work with Muir peach dwarf (4), observed the "stunt" disease in the San Joaquin County orchard in 1952 and concurred that its symptoms were not identical with those of Muir peach dwarf. Recently, Milbrath (8,9,10,11) suggested that peach stunt may be part of the ring spot virus complex which is common to many species of Prunus. Regardless of the correct name for the disease, peach stunt is recognized as an economically important disease of peaches and nectarines in California.

SYMPTOMS AND EFFECTS

The most conspicuous symptom is dwarfed twig growth on which small, dark green leaves are arranged close together as a result of shortened internodes. The rosetted leaves sometimes are small and narrow, and often undulated. On some occasions the small apical leaves show a conspicuous twist. The shortened internodes and rosetted arrangement of the leaves are most conspicuous in May. As the growing season advances through the summer, the newly formed internodes are more normal in length and the affected trees become less conspicuous.

Affected twigs tend to produce more leaf buds with a reduction in the number of flower buds. No flower or fruit symptoms associated with this disease have been noted, other than a reduction in the number of flower buds and, consequently, in the amount of fruit.

The distribution of virus symptoms in individual trees is variable. In some trees only a few of the branches show virus symptoms, whereas in others the entire tree exhibits symptoms of the disease. The damage caused by this disease is not sudden, but is gradual; the deleterious effect increases with each year of infection. Advanced cases of the disease show premature decline of the trees, with scattered terminal growth dying, much as reported for Muir peach dwarf.

---

[1]Most of the information in this paper was obtained through work done under Agricultural Marketing Service Project number SDA-Calif. -A-2, for which State funds were matched with Federal funds received from the Agricultural Marketing Service, United States Department of Agriculture, under provisions of the Agricultural Marketing Act of 1946.

[2]Respectively, Plant Pathologist, former Assistant Plant Pathologist, and Plant Pathologists, Bureau of Plant Pathology, California Department of Agriculture, Sacramento.

[3]Thanks are extended to Mr. Paul Jorgensen, Plant Pathologist, San Joaquin County Department of Agriculture, for assistance in making this survey.

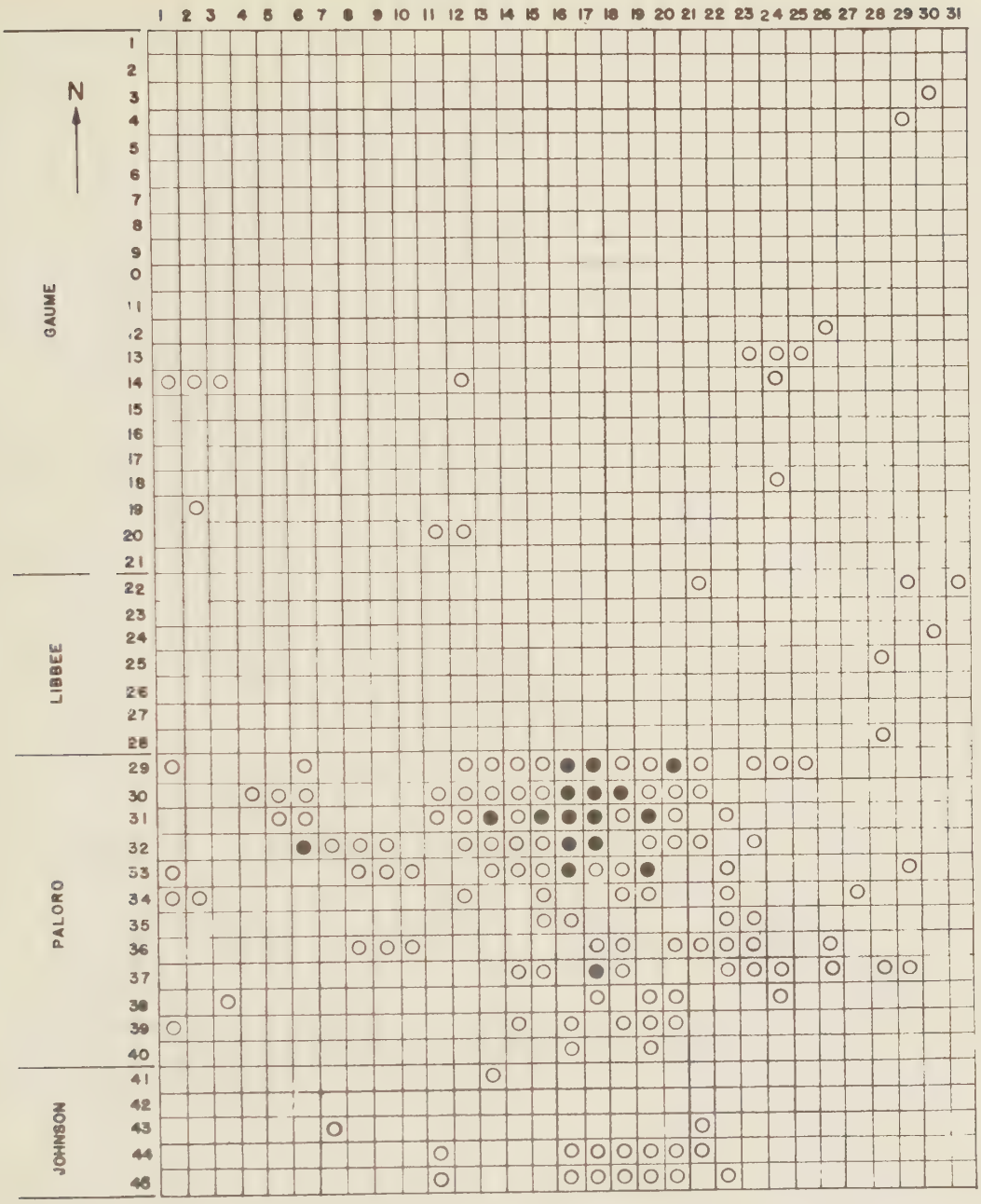


FIGURE 1. Schematic drawing of 14-acre peach planting showing arrangement of the varietal blocks and distribution of infected trees. Each square represents a tree position. Original infected trees found in 1952 are designated by the solid circles, whereas new cases resulting from spread between 1952 and 1959 are indicated by open circles. (Prepared by Sam Gotan)

DISTRIBUTION AND SPREAD

Subsequent to discovery of the disease in San Joaquin County, it was observed in various orchards and in growing nursery stock in several California counties (2, 3, 5, 7, 13, 14). Because of the severity of the disease, all affected nursery stock was destroyed (2, 3, 5, 6, 7). The occurrence of the disease in nursery stock in several counties indicates that infected propagating wood has served to perpetuate it. It is of interest that in California nursery stock the disease has been seen, and diagnosed by indexing, only in yearling trees. Although suspected

in June-budded growing nursery stock on several occasions, index tests have failed to confirm presence of the disease. Recognition of the disease on June-budded stock in the nursery row is difficult because the shortened internodes so conspicuous on affected twigs during early spring growth are less pronounced on the growth made during higher temperatures of the summer months, the period when June-budded stocks make their greatest growth.

The 1959 survey of the 14-acre planting revealed 152 tree cases of the disease including 114 Paloro, 17 Johnson, 15 Gaume, and 6 Libbee trees (Fig. 1). This apparent spread suggests that a vector is involved. The largest number of new cases was in the Paloro and Johnson blocks, which together account for 17 of the 45 rows comprising the 14 acres. Apparently the causal virus spread more rapidly toward the south than toward the north. The prevailing winds are from the north and west. This virus, or related viruses causing similar symptoms in peach, does not appear to be present in other stone fruits in California. Stunt symptoms have been observed in peach trees when inoculated with buds from individual Burbank and Royal Ann (Napoleon) sweet cherry, Montmorency sour cherry and Beni Higan flowering cherry trees. The symptoms so characteristic in peach were not noted in these cherry trees. No attempt was made to transmit the virus in the Paloro planting to Italian prune; however, several other cultures of peach stunt in California have been inoculated into Italian prune and the prune trees developed symptoms of the prune dwarf virus disease. This is of interest in that there have been only four tree cases of naturally occurring prune dwarf reported in plums and prunes in California (1). Apparently not all of the factors necessary for the natural spread of the prune dwarf virus to prunes are widely present in California. Reservoirs of the virus do exist in stone fruits adjacent to susceptible domestica plum tree plantings, but natural spread to them evidently has not occurred to any great extent.

Literature Cited

1. COCHRAN, L. C. 1956. Prune dwarf expression in plum varieties. (Abst.) Phytopathology 46: 635.
2. HILTABRAND, W. 1953. Nursery Service in 34th Annual Rept., Calif. Dept. Agr. Bull. 42: 246-252.
3. HILTABRAND, W. F. 1958. Nursery Service in 38th Annual Rept., Calif. Dept. Agr. Bull. 47: 193-197.
4. HUTCHINS, L. M., C. F. KINMAN, L. C. COCHRAN and G. L. STOUT. 1951. Muir peach dwarf in Virus diseases and other disorders with viruslike symptoms of stone fruits in North America. U. S. Dept. Agr. Handb. 10, pp 63-70.
5. MESSENGER, A. P. 1954. Bureau of Plant Quarantine, in 35th Annual Rept., Calif. Dept. Agr. Bull. 43: 197-208.
6. MESSENGER, A. P., and E. A. BREECH. 1956. Bureau of Plant Quarantine in 36th Annual Rept., Calif. Dept. Agr. Bull. 45: 188-198.
7. MESSENGER, A. P., and E. A. BREECH. 1957. Bureau of Plant Quarantine in 37th Annual Rept., Calif. Dept. Agr. Bull. 46: 183-190.
8. MILBRATH, J. A. 1956. Squash as a differential host for strains of stone fruit ring spot viruses. (Abst.) Phytopathology 46: 638-639.
9. MILBRATH, J. A. 1957. The relation of peach ring spot virus to sour cherry yellows, prune dwarf, and peach stunt. (Abst.) Phytopathology 47: 529.
10. MILBRATH, J. A. 1957. Effect of sour cherry viruses on growth of young orchard trees. Phytopathology 47: 655-657.
11. MILBRATH, J. A. 1959. Mechanical transmission of the virus responsible for sour cherry yellows, prune dwarf, and peach stunt. (Abst.) Phytopathology 49: 545.
12. REEVES, E. L. 1951. Stone fruit virus problems in the Northwest. 43rd Annual Rept., Ore. State Hort. Soc. pp 119-123.
13. WAGNON, H. K. 1953. Virus-free nursery stock in 34th Annual Rept., Calif. Dept. Agr. Bull. 42: 267-270.
14. WAGNON, H. K. 1954. Virus-free nursery stock in 35th Annual Rept., Calif. Dept. Agr. Bull. 43: 220-223.

BUREAU OF PLANT PATHOLOGY, CALIFORNIA DEPARTMENT OF AGRICULTURE, SACRAMENTO

VEGETABLE DISEASES IN NORTH CAROLINA DURING 1958 AND 1959

N. N. Winstead, D. L. Strider, and L. H. Person

The relative importance of many vegetable diseases has changed since Ellis reported on the occurrence of diseases of vegetables in North Carolina in 1942, 1946, 1951, and 1953 (1, 2, 3, 4). The diseases, their relative severity and distribution within North Carolina in 1958 and 1959 are listed in Table 1.

BEAN -- In the mountain area of North Carolina in 1959 an unusually wet season intensified many diseases of the late-summer bean crop. In this area about one-fifth of the 12,000 acres of snapbeans is planted to pole bean varieties, primarily Kentucky Wonder 191. Wade is the predominant bush bean variety. Growers of late-season snapbeans experienced heavy losses from two fungus diseases. Approximately 80% of the pole beans were destroyed by rust before harvest. In all plantings rust caused moderate to severe losses and in many plantings there was a total loss. Disease severity was emphasized by the absence of rust-free leaves; moreover, numerous pustules were found covering pods, petioles, and even the stems. Bush beans, although usually infected, were less severely affected than pole bean. Angular leaf spot was especially destructive on late plantings of bush beans in this area. Some plantings were severely defoliated, and it was estimated that growers harvested only half a normal crop.

In Eastern North Carolina bean plants were affected with a leaf spot disease not previously observed here. Spots were small, angular, and somewhat grayish in color. Perithecia containing ascospores were observed within the lesions. The disease caused losses of approximately 20%. The causal fungus produced both conidia and ascospores in culture and was tentatively identified as Pleospora herbarum. No pathogenicity tests were made; however, this fungus has been reported as causing a leaf spot of bean (5, 8).

In all bean-growing areas root rot diseases were especially prevalent in plantings in both 1958 and 1959. In most fields root rot was associated with two or more pathogens and losses ranged from only a trace to 50%. Fungi most frequently associated with diseased plants were Sclerotium rolfsii, Rhizoctonia solani, and Fusarium spp.

Several nematodes, Pratylenchus penetrans, Tylenchorhynchus claytoni, Meloidogyne hapla, and M. incognita acrita, were associated with stunted bean plants and appeared to cause considerable damage in eastern North Carolina.

CABBAGE -- Black rot was much more prevalent and destructive in both eastern and western North Carolina in 1958 and 1959 than it had been for the past several years. Yellows was of considerable importance on susceptible varieties in western North Carolina and on the fall crop in eastern North Carolina. Damping off, wire stem, and bottom rot were widely distributed. Damping off was particularly destructive in eastern North Carolina on the fall crop where it was seeded directly in the field. Other major diseases were downy mildew and root knot. The nematode species most frequently associated with root knot was M. incognita acrita. Clubroot was probably the most destructive disease of cabbage in the mountain area.

CARROTS -- Carrots planted at the Mountain Horticultural Crops Research Station, Fletcher, North Carolina in 1959 were severely infected with Alternaria dauci, rendering the crop a complete loss. Yields of several varieties included in trial plantings at the Coastal Plains Vegetable Station, Faison were reduced 50 % or more by Sclerotium rolfsii.

CUCURBITS

Because of the availability of commercially acceptable varieties which exhibit resistance to one or more diseases, major changes have occurred in the relative importance of the various cucurbit diseases. Downy mildew, formerly considered one of the most important diseases, caused little damage in 1958 and, except for fall cucumbers, none in 1959. Although it has continued to be of some importance on squash and pumpkin and, to a lesser extent, on fresh market cucumbers in the mountain area, the widespread use of resistant cucumber and muskmelon varieties has delayed the appearance and decreased the severity of this disease in North Carolina. Similarly, the use of resistant watermelon varieties during the past 7 years has reduced the incidence of anthracnose and wilt so markedly that they are now considered to be of minor importance.

CUCUMBER -- During the past 2 years anthracnose has decreased in importance, partly because of the use of Western-grown and $HgCl_2$-treated seed as well as the use of rotations with non-cucurbit crops. Where these practices have not been followed, complete crop losses have often occurred.

Angular leaf spot was more prevalent and destructive in 1959 than in any year since farmers began planting seed produced in the arid regions of western United States.

Table 1. Distribution and relative importance of certain diseases of bean, cabbage, carrot, cucurbits, eggplant, onion, pepper, southern pea, and tomato in North Carolina in 1958 and 1959.

Crop and disease	Causal organism	Distribution	Importance[a]
POLE BEAN			
Rust	Uromyces phaseoli (Pers.) Wint. var. typica Arth.	Mountains	Major on late summer crop
BUSH BEAN			
Angular leaf spot	Isariopsis griseola Sacc.	Mountains	Major on late summer crop[b]
Ascochyta leaf spot	A. phaseolorum Sacc.	Mountains	Minor[c]
Ashy stem blight	Macrophomina phaseoli (Maubl.) Ashby	Coastal Plain	Minor[b]
Fusarium root rot	Fusarium spp.	All areas	Major
Leaf spot	Pleospora herbarum (Pers. & Fr.) Rab.	Coastal Plain	Minor[d]
Nematodes - Meadow	Pratylenchus penetrans (1917) Filipjev & Stekhoven, 1941	All areas	Minor[d]
Nematodes - Root-knot	Meloidogyne incognita acrita, Chitwood, 1949, M. hapla, Chitwood, 1949	Coastal Plain	Minor[d]
Nematodes - Soybean cyst	Heterodera glycines Ichinohe, 1952	Coastal Plain	Minor[b]
Nematodes - Stunt	Tylenchorhynchus claytoni Steiner, 1937	Coastal Plain	Minor[d]
Phyllosticta leaf spot	P. phaseolina Sacc.	All areas	Minor[c]
Pythium wilt	P. ultimum Trow	Coastal Plain	Minor[d]
Rhizoctonia root rot	R. solani Kuehn	All areas	Major
Rust	U. phaseoli var. typica	Mountains	Minor[c]
Southern blight	Sclerotium rolfsii Sacc.	Piedmont & Coastal Plain	Major
Web blight	Rhizoctonia microsclerotia Matz.	Coastal Plain	Minor[b]
CABBAGE			
Black rot	Xanthomonas campestris (Pam.) Dowson	All areas	Major
Clubroot	Plasmodiophora brassicae Wor.	Mountains	Major
Downy mildew	Peronospora parasitica (Pers.) Fr.	All areas	Major
Root-knot	Meloidogyne incognita acrita	Coastal Plain	Major
Wire stem, damping off and bottom rot	Rhizoctonia solani	All areas	Major
Yellows	F. oxysporum Schlecht. f. conglutinans (Wr.) Snyder & Hansen	All areas	Major
CARROT			
Alternaria leaf spot	A. dauci (Kuehn) Groves & Skolko	Mountains	Major
Southern blight	S. rolfsii	Coastal Plain	Major
CUCURBITS			
CUCUMBER			
Angular leaf spot	Pseudomonas lachrymans (E. F. Sm. & Bryan) Carsner	All areas	Minor[d]
Anthracnose	Colletotrichum lagenarium (Pass.) Ell. & Halst.	All areas	Major[d]
Bacterial wilt	Erwinia tracheiphila (E. F. Sm.) Holland	Mountains	Moderate
		Piedmont & Coastal Plain	Minor[c]
Cercospora leaf spot	C. citrullina Cke.	Coastal Plain	Minor[c]
Cottony leak	Pythium aphanidermatum (Edson) Fitz.	Coastal Plain	Major
Downy mildew	Pseudoperonospora cubensis (Berk. & Curt.) Rostow.	Coastal Plain	Minor (season over before disease developed)
		Piedmont	Moderate
		Mountains	Moderate
Fruit rot	Rhizoctonia solani	All areas	Moderate
Root-knot	M. incognita acrita	Coastal Plain	Major
Scab	Cladosporium cucumerinum Ell. & Arth.	Mountains	Major
MUSKMELON			
Alternaria leaf spot	A. cucumerina (Ell. & Ev.) J. A. Elliott	Coastal Plain & Piedmont	Major
Bacterial wilt	E. tracheiphila	Coastal Plain & Piedmont	Minor[c]
Cercospora leaf spot	C. citrullina	Coastal Plain & Piedmont	Minor[c]
Downy mildew	P. cubensis	Coastal Plain & Piedmont	Minor (season over before disease developed)
Fruit rot	Fusarium spp.	Coastal Plain & Piedmont	Moderate
Fruit rot	Phytophthora spp.	Coastal Plain & Piedmont	Moderate
Fruit rot	Pythium spp.	Coastal Plain & Piedmont	Moderate
Fruit rot	R. solani	Coastal Plain & Piedmont	Moderate
Fruit rot	S. rolfsii	Coastal Plain & Piedmont	Major
Gummy stem blight	Mycosphaerella melonis (Pass.) Chiu & J. C. Walker	Coastal Plain & Piedmont	Major
Powdery mildew	Eryisphe cichoracearum DC.	Coastal Plain & Piedmont	Minor[d]
Root-knot	M. incognita acrita	Coastal Plain	Major
SQUASH			
Choanephora fruit rot	C. cucurbitarum (Berk. & Rav.) Thaxt.	All areas	Moderate
Downy mildew	P. cubensis	Mountains	Major
Mosaic	Undetermined	All areas	Minor[d]
Phytophthora blight	P. capsici Leonian	Coastal Plain	Minor[d]
Powdery mildew	E. cichoracearum	All areas	Moderate
Scab	Cladosporium cucumerinum	Mountains	Major

Table 1 (Cont.)

Crop and disease	Causal organism	Distribution	Importance[a]
WATERMELON			
Anthracnose	Colletotrichum lagenarium race 1	Coastal Plain & Piedmont	Moderate[d]
Anthracnose	C. lagenarium race 2	Coastal Plain & Piedmont	Minor[d]
Cercospora leaf spot	C. citrullina	Coastal Plain & Piedmont	Minor[c]
Fruit rot	S. rolfsii Sacc.	Coastal Plain & Piedmont	Major
Fusarium wilt	F. oxysporum (Schlecht.) f. niveum (E. F. Sm.) Snyder & Hansen	Coastal Plain & Piedmont	Minor[d]
Gummy stem blight	Mycosphaerella melonis	Coastal Plain & Piedmont	Major
Root-knot	Meloidogyne incognita acrita	Coastal Plain & Piedmont	Major
EGGPLANT			
Anthracnose	Gloeosporium melongenae Ell. & Halst.	Coastal Plain	Minor[b]
Bacterial wilt	Pseudomonas solanacearum E. F. Sm.	Coastal Plain	Major
Phomopsis blight	P. vexans (Sacc. & Syd.) Harter	Coastal Plain	Minor[d]
Southern blight	S. rolfsii	Coastal Plain	Moderate
ONIONS			
Pink root	Pyrenochaeta terrestris (Hansen) Gorenz, J. C. Walker & Larson	Coastal Plain	Major
Purple blotch	Alternaria porri (Ell.) Cif.	Coastal Plain	Major
Root-knot	Meloidogyne spp.	Coastal Plain	Minor[b]
Seedling root rot	Pleospora herbarum	Only on seedlings	Minor
Southern blight	S. rolfsii	Coastal Plain	Minor[d]
PEPPER			
Anthracnose	Colletotrichum capsici (Syd.) Butl. & Bisby	All areas	Major on ripe pepper
Anthracnose	C. nigrum Ell. & Halst.	All areas	Major on ripe pepper
Anthracnose	Gloeosporium piperatum Ell. & Ev.	All areas	Major on ripe pepper
Ascochyta leaf spot	A. phaseolorum	All areas	Minor[c]
Bacterial spot	Xanthomonas vesicatoria (Doidge) Dows.	All areas	Major
Bacterial soft rot	Erwinia spp.	Coastal Plain	Minor
Cercospora leaf spot	C. capsici Heald & Wolf	All areas	Major in processing crop
Phytophthora blight	P. capsici	Coastal Plain	Minor[d]
Southern blight	S. rolfsii	Coastal Plain	Major
SOUTHERN PEA			
Ascochyta blight	A. phaseolorum	Mountains	Minor[c]
Bacterial blight	Xanthomonas vignicola Burkh.	All areas	Minor[d]
Cladosporium spot	C. vignae Gardner	Mountains	Major
Fusarium wilt & root rot	Fusarium spp.	Piedmont & Coastal Plain	Moderate
Mosaic	Undetermined	Piedmont	Minor[d]
Pod rot	Choanephora cucurbitarum	All areas	Moderate
Root-knot	M. incognita acrita	Coastal Plain	Moderate
Southern blight	S. rolfsii	Coastal Plain	Moderate
Target spot	Corynespora cassiicola (Berk. & Curt.) Wei	All areas	Moderate
TOMATO			
Anthracnose	Colletotrichum phomoides (Sacc.)	All areas	Minor[d]
Ascochyta leaf spot	A. phaseolorum	Mountains	Minor[c]
Bacterial canker	Corynebacterium michiganense (E. F. Sm.) Jensen	Mountains	Minor[d]
Bacterial spot	Xanthomonas vesicatoria	All areas	Major
Bacterial wilt	P. solanacearum	All areas	Major
Botrytis blight	B. cinerea (Pers.) Fr.	Greenhouses only	Minor[d]
Early blight	Alternaria solani (Ell. & Mart.) L. R. Jones & Grout	All areas	Major
	Alternaria tomato (Cke.) G. F. Weber	All areas	Moderate
Fruit rot	Rhizoctonia solani	All areas	Major
Fusarium wilt	F. oxysporum Schlecht. f. lycopersici (Sacc.) Snyder & Hansen	All areas	Major
Gray leaf spot	Stemphylium solani G. F. Weber	All areas	Major
Late blight	Phytophthora infestans (Mont.) DBy.	All areas	Minor
Mosaic	(Primarily tobacco mosaic)	All areas	Major on staked tomatoes
Root-knot	Primarily M. incognita acrita	All areas	Major
Southern blight	S. rolfsii	Coastal Plain	Major on plants and as fruit rot

[a]Relative importance in area concerned.
[b]Observed in only a few fields and of minor importance in these.
[c]Widespread but of minor importance.
[d]Observed in a few fields but destructive in these fields.

Scab was very severe in the mountain areas of North Carolina in 1959. In most of the fields few scab-free fruits could be found. Bacterial wilt was generally distributed in eastern North Carolina early in the season and in a few cases stands were reduced. In the mountains it was prevalent throughout the growing season.

MUSKMELON -- Several foliage diseases were present on muskmelon in both 1958 and 1959. In many fields several varieties were planted and the diseases varied with variety. Alternaria leaf spot and gummy stem blight were the two most important foliage diseases. All varieties grown in North Carolina were highly susceptible to the gummy stem blight fungus, and this disease was the most serious foliage disease during 1958 and 1959. The Alternaria disease was especially widespread on Rio Gold and to a slightly lesser extent on the Hales Jumbo and Hales Best varieties. The Edisto and Smith's Perfect varieties appeared to be very resistant to A. cucumerina. Rio Gold, Edisto, and Smith's Perfect were all resistant to downy mildew; however, powdery mildew was frequently severe on Smith's Perfect but not on Rio Gold and Edisto which were resistant. Virus diseases were encountered infrequently except on Smith's Perfect. The specific virus concerned was not identified.

The disease which caused greatest economic loss to muskmelon growers during the past 2 years was a fruit rot incited by Sclerotium rolfsii. Field surveys in 1959 indicated that these losses approximated 15%.

SQUASH -- Powdery mildew, mosaic and a fruit rot incited by Choanephora cucurbitarum were distributed throughout the State. Scab and downy mildew were serious only in the mountains, while Phytophthora blight was more important in eastern North Carolina.

WATERMELONS -- Anthracnose continued to be very destructive on susceptible varieties and was observed infrequently on resistant varieties when C. lagenarium race 2 (7) was present. Thus far race 2 did not appear to have become widespread. Fusarium wilt also was quite destructive on susceptible varieties and was generally present in soils in all areas. Severe wilt (85% of the plants killed) was observed twice on Charleston Gray watermelons where root knot (M. incognita acrita) was severe.

Gummy stem blight was the most destructive disease of watermelon during the past 2 years. It was widespread and in many instances was responsible for complete crop losses.

EGGPLANT -- Losses from Phomopsis blight during the past several years have been reduced markedly in comparison with those in earlier years. This is undoubtedly associated with the development of resistant varieties such as Florida Market, Florida High Bush, and Fort Myers Market. In the past 2 years bacterial wilt was by far the most important disease on this crop. Losses in excess of 50% was common. Anthracnose was observed in 1959 on fruits in an experimental planting of the bacterial wilt resistant varieties, Matale and Kopek. The pathogen was tentatively identified as Gloeosporium melongenae.

ONION -- During the past 4 years there has been considerable interest in the growing of onions in southeastern North Carolina. Prior to the passage of the North Carolina Plant Law in 1959, little effort was made to prevent the importation of diseased transplants; hence, a number of disease problems previously unknown in North Carolina became extremely important. In 1958 pink root was observed in almost all onion fields. In 1959 the disease was severe in all fields which had been planted to onions in either 1957 or 1958, and traces of the disease were observed in almost all fields, even those never previously planted in onions. In 1959 yellow dwarf was quite severe in several fields and yields were reduced appreciably. In 1958 purple blotch almost completely defoliated onion plants on 50% of the acreage. A fungus tentatively identified as Pleospora herbarum has been isolated from dead roots of onion seedlings. The fungus produced Stemphylium conidia in culture and mature perithecia in dead roots as well as in culture.

Sclerotium rolfsii was particularly destructive on white onions. Little or no damage by this fungus was observed on onions with colored skins except on bulbs having splits or mechanical injuries in the outer scales. In 1958 a field of onions was observed which contained both white and colored varieties. By harvest time 75% of the white bulbs were infected with S. rolfsii whereas less than 1% of the colored bulbs in adjacent rows were affected, suggesting that the colored onions might be resistant to S. rolfsii.

PEPPER -- Bacterial spot, the most destructive disease of pepper during the past 2 years, caused an estimated 15% loss of the North Carolina pepper crop in 1959.

Several recent developments may help to lessen the importance of this disease in the future. In 1959 the North Carolina Legislature amended the State Seed Law to the effect that pepper seed to be sold in North Carolina must either have been produced in the arid regions of western United States or have been treated with bichloride of mercury. Tests at the Coastal Plain Vegetable Crops Research Station, Faison, North Carolina during an 8-year period indicated that the causal bacterium overwinters in plant refuse approximately 1 out of every 3

years under field conditions. It did not survive under these conditions for more than 1 year; hence, crop rotations with nonsusceptible crops must also be followed. In 1959 the Legislature also passed a law establishing standards for the certification of transplants of a number of crops. These standards as defined by the North Carolina Department of Agriculture allow no tolerance for bacterial spot or other injurious diseases of pepper.

In 1959 new liquid formulations of an emulsifiable concentrate of a copper organic salt (Tennessee Corporation, 3% metallic copper (TC11) and 6% metallic copper (TC 22)) were used on an experimental basis for the control of bacterial spot. In this test the spray program was initiated in a pepper field where 95 to 100% of the foliage of all plants was infected. Control under these conditions with the new liquid copper formulation was much better than that obtained with a combination of copper (Tribasic Copper Sulfate 52% -- 4-100) plus streptomycin (200 ppm). This material offers considerable hope that the disease can be controlled by field application of chemicals.

Bacterial soft rot has caused losses to some growers, especially where fruits were washed and stored under hot, humid conditions one or more days before sale. In such cases a bacterial soft rot developed most frequently in the wounded portions of the fruit stems.

SOUTHERN PEA -- Few reports on the Cladosporium spot disease of pea, Vigna sinensis, have been published since it was first recorded by Gardner in 1925 (6). The growers of peas in western North Carolina experienced heavy losses from this disease during 1959, and many acres of this crop failed to produce any marketable pods.

Target spot has been quite prevalent throughout the State and has caused complete defoliation in many cases.

A mosaic, seed-borne virus disease also was observed on a blackeye type pea in the Piedmont area of North Carolina. In this case the grower had saved his own seeds. In greenhouse tests approximately 2% of the plants grown from seed obtained from the grower showed mosaic symptoms. No attempt has been made to identify the virus concerned.

TOMATO -- Tobacco mosaic is frequently important, especially on staked tomatoes. A gray-wall type disease, and Botrytis foliage and fruit blight was of some importance in greenhouses. Anthracnose was important only on ripe fruits grown for processing or as a market disease.

Such diseases as early blight incited by Alternaria solani and to a lesser extent Alternaria tomato, gray leaf spot, Fusarium wilt, southern bacterial wilt, southern blight, and root knot were quite severe in both 1958 and 1959. Fruit rot pathogens, especially Sclerotium rolfsii and Rhizoctonia solani, were quite severe on unstaked tomatoes in 1959.

In 1958 bacterial canker was observed for the first time in a number of years at several locations in the mountains of North Carolina. Ascochyta blight was observed in almost all fields in western North Carolina in 1958. In one 7-acre field it caused a complete crop failure. This was the first known case where this disease has caused economic loss in North Carolina.

Literature Cited

1. ELLIS, D. E. 1942. Notes on plant diseases in North Carolina in 1941. Plant Disease Reptr. 26: 112-113.
2. ELLIS, D. E. 1951. Noteworthy diseases of cucurbits in North Carolina in 1949 and 1950. Plant Disease Reptr. 35: 91-93.
3. ELLIS, D. E. 1953. Cucurbit diseases in North Carolina and their control. North Carolina Agr. Exp. Sta. Bull. 380, 11 pp.
4. ELLIS, D. E., and R. S. COX. 1946. Notes on some vegetable diseases in North Carolina in 1946. Plant Disease Reptr. 30: 458-460.
5. DOYER, L. C. 1941. De Beteekinis van het zood als overbrenger van ziekten en plagen in groentegewassen. Tijdschr. over Plantenziekten 47: 14-24.
6. GARDNER, M. W. 1925. Cladosporium spot of cowpea. Phytopathology 15: 453-562.
7. GOODE, M. J. 1958. Physiological specialization in Colletotrichum lagenarium. Phytopathology 48: 79-93.
8. WEISS, F., and M. J. O'BRIEN. 1952. Index of Plant Diseases in the United States. U. S. Bur. Plant Indus., Soils, and Agr. Engin. Plant Disease Survey Spec. Publ. 1 (Part 4): 551-806.

DEPARTMENT OF PLANT PATHOLOGY AND CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, NORTH CAROLINA STATE COLLEGE, RALEIGH, NORTH CAROLINA

VARIATION IN MAIZE SEEDLING BLIGHT SYMPTOMS WITH CHANGES IN PATHOGEN SPECIES, ISOLATE AND HOST GENOTYPE[1]

Graydon C. Kingsland[2] and Clifford C. Wernham[3]

Summary

Fusarium moniliforme Sheld., Fusarium moniliforme Sheld. variety subglutinans Wr. & Rg. and Gibberella zeae (Schw.) Petch were isolated frequently from corn seedling axis and root tissue in Pennsylvania. A number of isolates of F. moniliforme and F. moniliforme var. subglutinans failed to cause seedling blight when introduced into pasteurized soil along with seeds of two inbred lines and two hybrids of corn. One isolate of G. zeae, on the other hand, caused severe blight on both inbreds and hybrids. A second isolate of G. zeae contributed to severe blight on the inbreds only. These results further demonstrate the pathogenicity of G. zeae, the variation of pathogenicity within the species, and the non-pathogenesis of several isolates of F. moniliforme and F. moniliforme var. subglutinans on corn seedlings under the experimental conditions employed.

INTRODUCTION

During cool spring weather seedling blight of corn frequently poses a serious problem. Gibberella zeae has been reported to be a serious offender in this respect by Branstetter (1), Manns and Phillips (6), Ho (3), Wenholz and Darragh (9), and Wernham (10). The roles of F. moniliforme and F. moniliforme var. subglutinans as incitants of seedling blight have not been clearly defined. Edwards (2) and Ullstrup (7) reported the latter to be pathogenic; F. moniliforme has been cited as an incitant of seedling blight of corn by Manns and Phillips (6), and by Valleau, Karraker, and Johnson (8). On the other hand, Holbert (4) and Leonian (5) have reported a wide range of pathogenicity between isolates of F. moniliforme.

During the course of research on the fungi associated with the stalk rot disease of corn in Pennsylvania, F. moniliforme, F. moniliforme var. subglutinans, and G. zeae were isolated from seedlings, some of which manifested symptoms of seedling blight. Experiments were conducted to determine whether the fungi obtained were pathogenic, and whether differences in pathogenicity exist between isolates.

MATERIALS AND METHODS

A 1:1 mixture of sand and silty clay loam soil was steamed for 45 minutes subsequent to reaching a temperature of 180° F. This soil was then placed in 56 eight-inch pots. Inoculum was prepared by growing two isolates of G. zeae, five isolates of F. moniliforme, and six isolates of F. moniliforme var. subglutinans on 8 ml of corn meal-dextrose agar in Petri dishes. Each isolate was then homogenized separately in 380 ml of sterile distilled water using a Waring Blendor. Fifteen seeds of the Pennsylvania hybrids 602A (Ind. WF9 X Oh. 51A) (Pa. 54 X Wis. 22), and Pennsylvania 775 (Ind. WF9 X Pa. 86)(Oh. 43 X Pa. 70), and the inbreds Pennsylvania 70 and Wisconsin 23 were placed in quarter sections of all 56 pots. Ninety ml of inoculum were poured around the seeds, which were then forced into the soil and covered. The checks received 90 ml of plain corn meal-dextrose agar homogenate. The pots were arranged at random in four replicates to minimize the effects of light, temperature and watering variation. In this arrangement each isolate plus one check occurred only once per replicate with each hybrid and inbred used in the experiment.

The seedlings were permitted to grow for 4 weeks, at which time each group was carefully removed from the soil, washed, and dried at 140° F for 24 hours. Dry weight averages per

---

[1] Contribution No. 261 from the Department of Botany and Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication as paper No. 2453 in the Journal Series.

[2] Formerly graduate research assistant in Botany and Plant Pathology, The Pennsylvania State University, University Park, Pa. Currently Pathologist, Department of Tropical Research, Tela R. R. Co. (A subsidiary of United Fruit Co.), LaLima, Honduras, C. A.

[3] Professor of Plant Pathology, Department of Botany and Plant Pathology, The Pennsylvania State University, University Park, Pa.

plant per plot replicate were obtained. These values were corrected to the equivalent of 10 plants to facilitate mathematical manipulation and subjected to an analysis of variance.

Isolations were made from the plants grown in soil seeded with G. zeae. No isolations were attempted from plants in soil seeded with F. moniliforme or F. moniliforme var. subglutinans, since these plants were as vigorous as the checks and there were no macrosymptoms of seedling blight present.

RESULTS

There was no reduction in dry weight yield or number of plants in either hybrids or inbreds grown in soil seeded with F. moniliforme or F. moniliforme var. subglutinans. The plants in all replicates of these treatments were as vigorous as the check plants for the duration of the experiment. None of the plants revealed any macroscopic symptoms of root or seedling axis infection by the isolates of F. moniliforme or F. moniliforme var. subglutinans employed.

The isolates of G. zeae varied in the observed effect on the corn used in this experiment. Isolate 1614 contributed to the complete blighting of all seedlings in both hybrids and inbreds, that is, no stand was obtained from seed in soil infested with this isolate. Isolate 1610, on the other hand, was not sufficiently virulent to significantly reduce the dry weights of hybrids in comparison with the checks. It did, however, cause complete blighting of the seedlings of both inbred lines. Initial stands of seedlings in soil seeded with G. zeae were poor in comparison with all other stands. In all cases, except G. zeae isolate 1610 on hybrids, the plants grew slower than the checks and manifested seedling blight symptoms during the entire course of the experiment. These symptoms included water-soaked leaf streaks and leaf tip chlorosis. Chlorotic areas ultimately became necrotic. Vigor of the plants was reduced greatly and black lesions developed on the mesocotyl and roots. All attempted isolations from seedlings in soil seeded with either isolate of G. zeae were successful.

The experimental results described further demonstrate the variability in pathogenicity between isolates of G. zeae. In selecting for seedling blight resistance, therefore, the practice of incorporating several isolates of this fungus into any screening program is desirable.

Literature Cited

1. BRANSTETTER, B. B. 1927. Corn root rot studies. Missouri Agr. Exp. Sta. Research Bull. 113. 80 pp.
2. EDWARDS, E. T. 1933. A new Fusarium disease of maize. Agr. Gaz. New South Wales 44: 895-897.
3. HO, WEN-CHUN. 1944. Soil inhabiting fungi attacking the roots of maize. Iowa Agr. Exp. Sta. Research Bull. 332. 483.
4. HOLBERT, J. R. 1924. Corn root, stalk and ear rot diseases. University of Illinois Agr. Exp. Sta. Bull. 255. 239-478.
5. LEONIAN, L. H. 1932. Pathogenicity and variability of Fusarium moniliforme from corn. West Virginia Agr. Exp. Sta. Bull. 248. 1-16.
6. MANNS, T. F., and C. E. PHILLIPS. 1924. Corn root rot studies. J. Agr. Research 27: 957-964.
7. ULLSTRUP, A. J. 1936. The occurrence of Gibberella fujikuroi var. subglutinans in the United States. Phytopathology 26: 685-693.
8. VALLEAU, W. D., P. E. KARRAKER, and E. M. JOHNSON. 1926. Corn root rot, a soil borne disease. J. Agr. Research. 33: 453-476.
9. WENHOLZ, H., and W. H. DARRAGH. 1927. Root, stalk and ear rot diseases in maize; suggestions for their control. Agr. Gaz. New South Wales 38: 39-49.
10. WERNHAM, C. C. 1951. Cold testing of corn. Pennsylvania Agr. Exp. Sta. Progress Report 47. 1-12.

PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY PARK

MAIZE SYNTHETICS FOR DISEASE RESISTANCE[1]

C. C. Wernham[2]

Summary

The uses and advantages of disease resistant synthetics of maize to breeders and plant pathologists is discussed, with particular emphasis on the method used to acquire the adaptability of local varieties and maintain the resistance of the original synthetic. Four new synthetics are described. The availability of disease resistant inbred lines for distribution is also discussed with reference to the requirements to be met on receiving them.

The numerous agronomic investigations involving maize synthetics has been briefly reviewed by Sprague (2). That synthetics are popular vehicles of maize germ plasm is attested to by the recent decision of the North Central Corn Conference to publish a catalogue of the existing lots, that all may benefit through their distribution.

Synthetics produced for the specific purpose of disease resistance are rather rare. Only six have been produced, three from this laboratory (6), two from Purdue (Synthetics A and B), and one from Corn and Sorghum Investigations, Cereal Crops Research Branch, Crops Research Division, United States Department of Agriculture. This latter synthetic is a double-double cross involving eight lines in the 800-900 maturity class. Under local conditions the unrelated Purdue synthetics showed a pollination range from 7/25 to 8/9 from a May 12, 1959 planting. The composition of the three original synthetics from this laboratory has been noted elsewhere (5).

Sprague (2, p. 277) indicates that with respect to leaf blights of corn "a satisfactory degree of resistance can be transferred by a backcrossing technique." Jenkins and Robert (1) have shown that some blight resistant inbreds have a greater potency as resistant parents than others and that the potency is not always parallel to the resistance rating of the individual line. The failure to produce blight resistant recoveries of some outstanding inbreds may be due to the unfortunate choice of a resistant parent. In the 15 years' experience of this writer, backcrossing as a technique is more likely to be successful if it is the delayed type (backcross, self, select, backcross, self, select, and so forth) and it is far more rewarding with diseases such as seedling blights and cold reaction, smut and stalk rot due to Gibberella zeae. The better situation would be repairing the faults of a high yielding blight resistant inbred by backcrossing, using the blight resistant inbred as the recurrent parent. It is too easy to lose blight resistance through dilution of chromosomal material.

The "disease resistant" synthetic obviates many of the weaknesses of specific breeding and has some distinct advantages. Disease is notoriously sporadic and selection in a natural environment is hazardous. One cannot select profitably for disease resistance without disease and even in programs designed specifically for the purpose (3) some weather cycles are disease defying. Where disease nurseries are an integral part of the segregation processes, the greater the number of years a culture is exposed, the more stable its disease reaction is likely to be. The equation - (epiphytotic conditions x time = inoculation pressure) expresses the idea quite succinctly. A satisfactory resistance in Iowa may be wholly inadequate in the Everglades region of Florida owing to differences in inoculation pressure. A synthetic should by-pass many of these weaknesses.

The advantages of the disease resistant synthetic are many and particularly so to the breeder who lacks phytopathological facilities. He is free to follow any method of breeding he wishes from the most conservative to the "avant-garde." With early testing there may be some pathological reservations (4) but no more so for resistant material than for any other kind. In addition a synthetic allows the breeder to practice the "art" of breeding, choosing and selecting those items which appeal to his esthetic sense, rather than accepting without question the product of someone else's choice. Moreover the breeder can be sure that the attrition rate of inbred lines will not be complicated by subsequent abandonment due to disease susceptibility.

There are advantages of disease resistant synthetics to pathologists. In a State like Pennsylvania there are many locally grown open pollinated varieties for which the chief claim is local

---

[1] Contribution No. 262 from the Department of Botany and Plant Pathology, The Pennsylvania Agricultural Experiment Station.

[2] Professor of Plant Pathology, Department of Botany and Plant Pathology, The Pennsylvania State University, University Park, Pennsylvania.

adaptability. Usually a few individuals per 1000 plants will show considerable resistance even to leaf blights, although some collections appear to have none at all. We have used preexisting disease resistant synthetics to capture this local adaptation. The method is applicable to other areas in the corn-growing world and will be revealed in the following paragraphs.

In 1955, up to 1000 plants of the open pollinated varieties and strains of "Early Butler", "College White Cap", early and late strains of "West Branch Sweepstakes", a longfellow flint (Kautz) and an unidentified yellow dent (Switzgable) were planted in a top crossing block and detasselled. The male parents were mixed plantings of the Early, Intermediate and Late synthetics.

The whole planting was treated as a disease nursery and inoculated with Helminthosporium spp, smut and stalk rot organisms. Prior to frost the detasselled plants most resistant to leaf blights were marked and at harvest time the more resistant entries of each variety were reselected for ear height, ear size and lodging. The 12 to 30 ears of each variety were shelled and the seed bulked. In this one operation the incidence of genes for resistance was being augmented while one-half the inheritance for local adaptation was conserved.

During 1956, 10 to 20 nursery rows of each top crossed variety or strain were planted in the disease nursery and selfed. Promising S_1 selections were carried to S_2 generation and again selected in 1957.

In 1958 the late "Sweepstakes-like" S_2 seed was planted in an isolation block and submitted to reinoculation. Again the most resistant plants were marked prior to frost, and at harvest time these were culled for ear type, ear height, lodging, smut, stalk rot and ear mold. The remaining ears, slightly more than 100 in number, were bulk shelled. This lot of seed is designated "Sweepstakes Synthetic." Seed is available to interested breeders. The Synthetic to-date has segregated about 2% of yellow ears per season. It has been open pollinated twice.

In 1959 the Early Butler, College White Cap, early Sweepstakes, Kautz flint and Switzgable dent selections bearing pollination dates on S_2 plants from July 25 to August 3 from a May 12 planting were all synthesized in a single isolation block. The seed was harvested and bulked. This lot of seed is designated "Early Synthetic No. 2" and is open pollinated once only.

Individual S_2 and S_3 ears of each component of this synthetic are available for distribution, in addition to the synthetic itself.

In 1955 remnant seed of early generation inbreds that we were able to self under the extreme drought conditions, and that later stood up well under the 7-inch rainfall accompanying the subsequent hurricane, was taken to South Africa. A series of three plantings, 11 days apart, enabled crosses to be made with a collection of H. turcicum resistant cultures of African origin. The same opportunity existed for a number of H. turcicum resistant lines supplied by M. T. Jenkins of the United States Department of Agriculture. Seed of these crosses was divided between the Natal Agricultural Research Institute and The Pennsylvania State University. In 1957 equal numbers of seed from each cross were planted in an isolation block and the plants inoculated in the same manner as the disease nursery. At harvest time selection was made for smut and lodging resistance. Due to day-length effects the hybrids were rather tall and high eared.

In 1958 the O. P. 1. was again planted in isolation, inoculated and reselected, care being taken to pick out highly resistant plants with the lowest ear placement. Seed was again bulked for the O. P. 2. generation.

In 1959 two lots of seed of this origin were planted. (A), O. P. 2. seed selected in 1958 and (B), O. P. 1. seed to compare with (A) to see how effective selection had been. Two lots of seed are available from these plantings.

1. O. P. 3. seed designated "South African-American synthetic O. P. 3."
2. O. P. 2. seed from (B) which consists of bulked seed from 94 standing plants harvested December 17, 1959 and designated "South African American Synthetic: Stiff-stalked selection." Seed is available in bulk or by individual ears.

The 1958 season at University Park was rather cool and rainy. Helminthosporium turcicum reached a severity not usually attained in the disease nursery and as a result certain inbred cultures of S_3 - S_6 maturity were selected as having outstanding resistance. Promising S_0, S_1 and S_2 lots were equally easy to select. Twenty-five seeds of cultures S_3 and beyond in the inbreeding process are available to interested breeders as long as the supply lasts. Each breeder desiring seed will be requested to submit a report to this department on the agronomic suitability of these cultures whenever this character is determined by yield tests.

Literature Cited

1. JENKINS, M. T., and ALICE L. ROBERT. 1959. Evaluating the breeding potential of inbred lines of corn resistant to the leaf blight caused by Helminthosporium turcicum. Agron. J. 51: 93-96.
2. SPRAGUE, G. F. 1955. Corn breeding. In Corn and Corn Improvement. Academic Press Inc. 221-292.
3. WERNHAM, C. C. 1949. Techniques for inoculating corn with disease-producing organisms. (Revised) The Pennsylvania State University. Agr. Exp. Sta. Report 5.
4. WERNHAM, C. C. 1952. Disease resistance and early testing of maize. Science 116: 57-58.
5. WERNHAM, C. C. 1953. The mass production of blight-resistant corn. Plant Disease Reptr. 37: 138-141.
6. WERNHAM, C. C. 1954. Blight-resistant synthetics of field corn. Maize Genetics Cooperation News Letter. 28: 30.

PENNSYLVANIA AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY PARK

INSECTICIDE TREATMENTS FOR APHID CONTROL IN RELATION TO SPREAD OF BARLEY YELLOW DWARF VIRUS[1]

E. A. Dickason, W. B. Raymer and W. H. Foote[2]

Summary

In experiments conducted with systemic and contact insecticides to control vectors of barley yellow dwarf virus in Hannchen barley, there was an initial suppression of aphids following treatments and, in some instances, a delay in virus infection. However, 100% of the plants eventually became infected in all experiments. There were no significant differences in barley yield or quality as a result of treatments. Aphid populations peaked and declined at the same time in both early- and late-seeded barley. Population densities in the late-seeded plots were several times greater than in early-seeded plots at the time of peak activity.

INTRODUCTION

Barley yellow dwarf virus was first identified in western Oregon in 1954 when it was found at various locations throughout the Willamette Valley. Losses from yellow dwarf were light during 1955 and 1956, but the virus apparently became widely distributed in native and cultivated grasses. Since that date, losses for all cereal crops in western Oregon have been estimated at 5% in 1957, 10% in 1958, and 25% in 1959.

In 1958 yellow dwarf was found for the first time in all of the major grain-growing areas of the State, but heavy losses have been confined to the western and northwestern areas of the State.

This paper reports research relating to control of barley yellow dwarf virus through control of aphids which spread the disease.

METHODS AND MATERIALS

Three experiments were conducted in Hannchen barley fields in western Oregon during 1959 in an attempt to control barley yellow dwarf virus through control of aphid vectors.

O, O-diethyl S-(ethylthio)methylphosphorodithioate (Thimet [=phorate])[3] and O, O-diethyl S-2-(ethylthio)ethylphosphorodithioate (Di-Syston) each were applied as 5% granular formulations at the rates outlined in Tables 1, 2, and 3. These materials were broadcast on the soil surface with a fertilizer spreader and lightly disced into the soil immediately prior to seeding. Seeds were treated with carbon formulations of these insecticides at the rates outlined in Table 2. Emulsions prepared from a mixture of O, O-diethyl S(and O)-2-(ethylthio)ethylphosphorothioates (demeton) and O, O-diethyl O-p-nitrophenyl phosphorothioate (parathion) emulsifiable concentrate formulations were applied as spray to the plants (Table 3).

Experiment No. 1 was located in a farmer's field adjacent to a field of winter wheat near Brooks, Oregon. The plots were 100 feet square and arranged in a randomized block design. Experiments No. 2 and 3 were located on the Hyslop Agronomy Farm near Corvallis, Oregon. A diverse variety of cereals and grasses are grown on this farm. The plots in Experiment No. 2 were 20 x 100 feet and completely randomized, and in Experiment No. 3 the plots were 30 x 150 feet and arranged in a randomized block design. Treatments were replicated three times in each experiment.

Aphid populations were counted at intervals from the time of their appearance in the experimental areas until they were no longer present. Counts were made of the insects present on the uppermost leaf of 100 plants taken at random in each plot. The number of alate and apterous forms were recorded for each leaf sampled.

The incidence of disease was determined by counting the number of plants showing typical

---

[1]Technical Paper No. 1319, Oregon Agricultural Experiment Station.

[2]Respectively, Assistant Professor, Department of Entomology, Assistant Professor, Department of Botany and Plant Pathology, and Professor, Department of Farm Crops, Oregon State College.

[3]Phorate has been designated as the common name for Thimet.

Table 1. Summary of results of yellow dwarf virus vector control experiments in Hannchen barley field seeded April 16, 1959. (Experiment 1) Brooks, Oregon. Data are average of three replications.

Treatment	Untreated	phorate granular	Di-Syston granular
Rate actual toxicant per acre	-	21.7 oz.	20 oz.
Aphids (% plants infested)[a]			
May 12	10.7	5.0	3.0
May 22	37.0	13.3	13.6
June 18	17.0	11.3	15.6
Virus (% plants infected)			
June 3	19.0	22.6	21.6
June 16	100	100	100
Yield (bushels per acre)[b]	19.8	22.1	20.9
Bushel weight[b]	48.3	48.5	48.3
Percentage "thins"[b]	34.3	31.8	32.3

[a]Infested with alatae or apterae or both.
[b]No significant differences.

Table 2. Summary of results of yellow dwarf virus control experiments in Hannchen barley field seeded April 22, 1959. (Experiment 2) Granger, Oregon. Data are average of three replications.

Treatment	Untreated	phorate granular	phorate seed treat.	Di-Syston granular	Di-Syston seed treat.
Rate actual toxicant per acre	-	15.2 oz.	-	20.4 oz.	-
Actual toxicant/100 pounds seed	-	-	7.04 oz.	-	8 oz.
Aphids (% plants infested)[a]					
May 7	11.6	8.5	3.0	8.0	2.0
May 19	22.3	20.0	12.5	15.3	10.0
May 28	30.0	26.5	14.0	18.3	13.5
June 6	40.3	44.0	27.5	30.3	25.0
June 23	40.0	44.0	51.0	34.0	45.0
July 10	1.0	2.0	0	2.6	1.0
Virus (% plants infected)					
June 1	15.6	13.5	13.0	14.3	14.0
June 9	22.6	22.0	19.0	19.6	18.5
June 23	100	100	100	100	100
Seeding rate (pounds/acre)[b]	158	158	110	158	110
Stand (plants/100' row)	1061	1187	873	1030	937
Yield (bushels per acre)[c]	32.5	26.5	29.1	37.1	29.7
Bushel weight[c]	48.5	48.3	47.4	49.1	48.4
Percentage "thins"[c]	43.0	44.8	46.5	38.6	42.3

[a]Infested with alatae or apterae or both.
[b]All plots seeded at same setting on machinery.
[c]No significant differences.

yellow dwarf symptoms in either 12 or 16 3-foot sections of row in each plot, depending on size of plot, and converted to percentage infestation based on the stand count.

Yield data were obtained by harvesting a 40-inch swath the length of each plot with a self-propelled small plot combine. Bushel weights were obtained and the percentage of thin kernels measured by the percentage of seeds passing through a 5 1/2 / 64 x 3/4 inch barley sieve.

RESULTS AND DISCUSSION

The results of the vector control experiments are outlined in Tables 1, 2, and 3. In an attempt to summarize the data, aphid populations were reduced to percentage of plants infested. There was an initial suppression of insect infestation from all treatments. In Experiment No. 1 (seeded April 16) this suppression was evident through the five-leaf stage (Table 1, May 22 aphid counts). In Experiment No. 2 (seeded April 22) only some treatments suppressed populations through the five-leaf stage (Table 2, May 28 aphid counts). In Experiment No. 3 (seeded May 13) suppression of infestation up to five-leaf stage was not as pronounced as in the previous

Table 3. Summary of results of yellow dwarf virus control experiments in Hannchen barley field seeded May 13, 1959. (Experiment 3) Granger, Oregon. Data are average of three replications.

Treatment	Untreated	phorate granular	Di-Syston granular	demeton spray[a]	parathion spray[a]	parathion spray[a,b]
Rate actual toxicant per acre	-	22.2 oz.	32.5 oz.	4 oz.	4 oz.	4 oz.[b]
Aphids (% plants infested)[c]						
May 25	9.6	8.3	4.3	9.6	9.6	9.6
June 2	13.3	9.6	10.6	4.6	6.0	4.6
June 15	76.0	69.3	55.6	63.3	62.3	67.6
June 16						26.6
June 28	91.6	92.6	87.3	88.0	90.6	74.0
June 13	1.6	2.0	2.3	2.6	1.0	3.3
Virus (% plants infected)						
June 23	18.6	16.0	16.6	19.6	18.6	24.6
July 6	100	100	100	100	100	100
Stand (plants/100' row)	871	909	1184	889	996	948
Ladybird larvae (per 100' row)	72	65	72	51	79	63

[a]Spray applied when plants were in second-leaf stage with third leaf appearing.
[b]These plots treated second time in five-leaf stage with 4 ounces actual toxicant.
[c]Infested with alatae or apterae or both.

experiments (Table 3, June 15 aphid counts). Infection of cereals with barley yellow dwarf virus prior to the five-leaf stage results in severe stunting, discoloration, and reduction in yield.

Disease incidence is summarized for various counting dates in Tables 1, 2, and 3. There was very little difference in percentage of virus infected plants between treated and untreated plots. The early disease counts, made at the time symptoms were first evident, did not reflect the insect control obtained at time of infection, or earlier in the season. Eventually 100% of the plants were infected in all plots. However, in some treated plots in each experiment symptoms of the disease were not so severe as in untreated plots. For example in Experiment 1, the phorate granular treated plot in the first replication was surrounded on three sides by the untreated barley field and on the fourth side by an untreated check plot. The plants in the treated plot were more vigorous and showed less foliage discoloration and stunting than plants in the surrounding untreated areas of the field, and yielded 20% more than the adjacent untreated check plot. This response to treatment appeared to be associated with a delay in time of infection. All of the plants were eventually infected, but the delay in infection apparently permitted the treated plants to become more mature and hence more resistant to the effects of the virus.

There were no significant differences in yield or in quality as determined by weight per bushel and percentage of "thins" between treated and untreated plots (Tables 1 and 2). Seed treatment with the systemic insecticides phorate or Di-Syston did not appear to have an adverse effect on germination. The lower stand counts in seed-treated plots in Experiment No. 2 (Table 2) were believed to be the result of different seeding rates of treated and untreated seeds. During preliminary control studies in 1958, phorate seed treatment delayed germination and reduced barley stands.

The dominant species of aphids collected from the experimental plots was the rose-grass aphid, Macrosiphum dirhodum (Walk.), followed by the apple-grain aphid, Rhopalosiphum fitchii (Sand.) and the English grain aphid, M. granarium (Kby.). All three species are vectors of barley yellow dwarf virus.

One possible source of spring migrants of the rose-grass and apple-grain aphids was from a Festuca arundinacea Schreb. planting. These aphids produced alate forms in the spring that migrated from the fescue at heading time. Based on aphid collections, it appeared these same aphids infested the seedling barley. Winter cereals also supported a complex of apterous and alate aphids that possibly were the source of vectors that infested spring cereals. However, a large population of aphids remained on winter cereals and, unlike the fescue planting, there was no abrupt migration period.

Population densities of alatae and apterae in treated plots are not treated separately in the summary tables. In general, populations of apterae were very similar to those in untreated plots (Fig. 1). The alatae in treated plots in Experiments 1 and 2 varied from those in untreated plots. In Experiment 2 alatae populations in most treated plots developed in varying densities up to twice the populations illustrated for June 30 in the untreated plots (Fig.1). In Experiment 3, alatae forms in most treated plots did not develop in greater numbers than in untreated

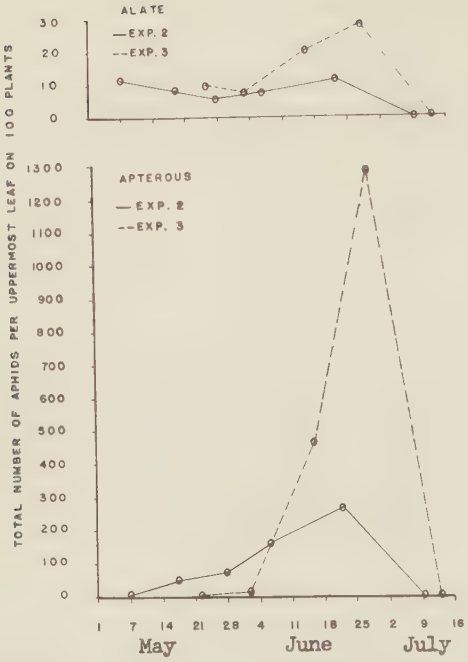


FIGURE 1

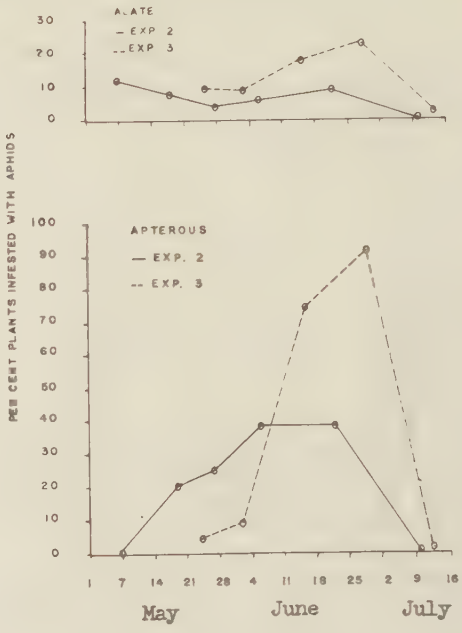


FIGURE 2

The effects of planting dates on the total number of alate and apterous aphids (Fig. 1), and the percentage of plants infested with these forms (Fig. 2). Data based on mean of three replications of untreated plots in each experiment. Experiment 2 seeded April 22, and Experiment 3 seeded May 13.

plots but they reached a peak population and began to decline approximately 2 weeks earlier than alatae in untreated plots. The abrupt decline of aphids (Figs. 1 and 2), and the differences discussed between treated and untreated plots, could not be related to the effects of insecticides on natural enemies. Parasites were rarely noted in any plots, and the low population of ladybird larvae was not affected by treatments (Table 3). Possibly higher temperatures or plant maturity had some effect on population decline, although during the 1958 season M. granarium was found on barley until harvest.

Under the conditions of Experiments 2 and 3 (which were in the same surroundings), there was a much lower population of alatae and apterae (Fig. 1) and a lower percentage of plants infested with these forms (Fig. 2) in the early-seeded field. The only apparent difference between these two experiments was the age of plants. The density of both alatae and apterae populations was several times greater in the late-seeded experiments, but reached a maximum peak and declined, independent of age of stand, during the same period in both experiments.

In both Experiments 2 and 3 there was 100% infection of the barley with virus, although there was a longer delay before this occurred in the earlier-seeded experiment. The relationship of increased aphid populations in late-seeded fields was also observed under general field conditions in western Oregon, and in some instances there was a marked relationship to the incidence of virus.

ACKNOWLEDGMENT

The writers acknowledge the cooperation of Miss L. M. Russell, Insect Identification and Parasite Introduction Research Branch, U.S.D.A., who identified the species of aphids collected during this study.

OREGON STATE COLLEGE, CORVALLIS, OREGON

CERCOSPORA BUNCHOSIAE A NEW LEAFSPOT DISEASE OF BARBADOS CHERRY

Harry C. Burnett[1] and J. R. McFarlin[2]

In December 1959 a leafspot of Barbados cherry, Malpighia glabra, was sent to the senior writer by Mr. J. R. McFarlin from a commercial nursery at Miami, Florida. The Cercospora found fruiting in the lesions was identified as Cercospora bunchosiae Chupp & Muller. Some of this material was sent to Dr. Charles Chupp of Cornell University, who confirmed the diagnosis. Figure 1 shows the disease on Barbados cherry.

FIGURE 1. Cercospora bunchosiae Chupp & Muller on Barbados cherry, Malpighia glabra (State Plant Board photo by Jerry Messec).

Infected leaves are deposited in the Florida Agricultural Experiment Station Herbarium under FLAS F 46876.

Chupp (1) and Chupp and Muller (2) report Cercospora bunchosiae only on Bunchosia glandulifera H. B. K. in Caracas, Venezuela.

The signs and symptoms of the disease on Barbados cherry are similar to those described by Chupp and Muller (2). The spots showing on both leaf surfaces are slightly sunken, circular or nearly so, dark brown with grey centers, surrounded by a yellow halo, 2 to 10 mm in diameter.

The morphology of the fungus conforms in most respects to the description of Chupp and Muller (2). The obclavate cylindric conidia 4.5-8 μ x 25-100μ are slightly longer than those found in B. glandulifera.

The fungus was cultured on potato-dextrose agar. Sporulation occurred on this medium within 30 days. A suspension of conidia and mycelial fragments from sporulating cultures was atomized on both leaf surfaces of Malpighia glabra. Symptoms first appeared in 10 days as slightly sunken yellow areas on both leaf surfaces. As the lesion enlarged, a dark brown margin appeared, surrounded by a narrow yellow halo.

Few diseases have been reported on Barbados cherry. Seymour (3) lists only Asterina indecora Syd. (Asterostomella indecora Syd.) and Meliola xenoderma Syd., while Weiss and O'Brien (4) only Cephaleuros virescens Kunze.

Literature Cited

1. CHUPP, C. 1953. A monograph of the fungus genus Cercospora. Published privately by the author. Ithaca, New York. 557 pp.
2. CHUPP, C., and A. S. MULLER. 1942. Bol. Soc. Venez. Cien. Nat 8 (52): 38.
3. SEYMOUR, A. B. 1929. Host Index of the Fungi of North America. Harvard University Press, Cambridge, Mass. 732 pp.
4. WEISS, FREEMAN, and MURIEL O'BRIEN. 1950-1953. Index of Plant Diseases in the United States. Plant Disease Survey Spec. Pub. 1.

STATE PLANT BOARD OF FLORIDA, WINTER HAVEN

1 Plant Pathologist, State Plant Board of Florida, Winter Haven, Florida.
2 District Inspector, State Plant Board of Florida, Miami, Florida.

BROAD SPECTRUM FUNGICIDES TESTED FOR CONTROL OF MELTING-OUT OF KENTUCKY BLUEGRASS AND SCLEROTINIA DOLLAR SPOT OF SEASIDE BENTGRASS[1]

Houston B. Couch and Laurence D. Moore[2]

Summary

In replicated field trials, nine fungicidal formulations were tested for control of melting-out of Kentucky bluegrass caused by Helminthosporium vagans. In addition, eight materials were tested for control of Sclerotinia dollar spot of Seaside bentgrass.

All of the materials tested significantly reduced the incidence of the leaf lesion phase of melting-out of Kentucky bluegrass -- Dyrene, Upjohn GAB-5, and California Spray ML-373, permitting faster plant recovery and, subsequently, denser stands of turfgrass.

Within 8 days from the time of first application, Tersan OM, Upjohn GAB-5, Acti-dione RZ, California Spray ML-372 and ML-373, and Dyrene reduced Sclerotinia dollar spot incidence from 75% to an average of 25% blighted turfgrass surface.

Melting-out, caused by Helminthosporium vagans Drechs., is the most common fungus disease of Kentucky bluegrass (Poa pratensis) in Pennsylvania. During periods of prolonged cool, wet weather the fungus can cause complete loss of bluegrass stands. The more typical disease pattern, however, is seen as thinning of stands due to a high order of leaf abscission. As the result, in addition to the immediate utilitarian and aesthetic losses, the plants thus weakened are more prone to the soon to follow crown and root rot phase of the disease (1).

Although generally recognized as an important disease of bentgrass (Agrostis spp.) putting greens and bowling greens, Sclerotinia dollar spot (Sclerotinia homoeocarpa F. T. Bennett) can also be quite severe on bluegrasses (Poa spp.) and fescues (Festuca spp.) used for home lawns, golf course fairways, and park areas. In these latter cases it is not uncommon in Pennsylvania for Sclerotinia dollar spot to be the predominant warm-weather, foliar fungus disease problem.

Several materials have been reported effective in controlling the leaf lesion phase of melting-out of Kentucky bluegrass (2, 3). These include Omadine (pyridine-2-thiol-1-oxide), Kromad, Acti-dione, captan, zineb, and phenyl mercury. Control of Sclerotinia dollar spot has generally been realized with the use of formulations containing cadmium (2, 4). Certain mercury-based materials, however, have also been reported to provide satisfactory levels of control (2, 5).

In the present investigation the following materials were tested for control of melting-out of Kentucky bluegrass: Dyrene (50% 2,4-Dichloro-6-o-chloroanilino-s-triazine), Upjohn GAB-5 (0.75% cycloheximide, 75% bis (dimethylthiocarbamoyl) disulfide), Acti-dione RZ (1.3% cycloheximide, 75% pentachloronitrobenzene), California Spray ML-372 (60% N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide, 5% cadmium carbonate, 10% bis (dimethylthiocarbamoyl) disulfide), California Spray ML-373 (60% N-trichloromethylthiophthalimide, 5% cadmium carbonate, 10% bis(dimethylthiocarbamoyl) disulfide), Hercules 3944-H (50% 5-chloro-4-phenyl-1,2-dithiole-3-one), and Hercules 4223-H (50% 5-chloro-4-p-tolyl-1,2-dithiole-3-one). In addition, two of the fungicides recommended for melting-out control, Orthocide 50-W (50%N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide), and Kromad (5% cadmium sebacate, 5% potassium chromate, 1% malachite green, 0.5% auramine, 16% bis (dimethylthiocarbamoyl) disulfide) were included as standards for comparison of quality of control (Table 1).

Each formulation was tested at two rates through one and three applications for a total of 31 treatments randomized in six replications (Table 1). Each plot measured 3 feet by 25 feet. Materials were applied under 35 pounds' pressure, using a boom-type system, equipped with Tee Jet flat spray tip nozzles, held 18 inches over the turf. All applications were made on the basis of dilution in 3.5 gallons of water per 1000 square feet of turfgrass.

In the Sclerotinia dollar spot control trials, Dyrene, Upjohn GAB-5, Acti-dione RZ, Hercules 3944-H and 4223-H, California Spray ML-372 and ML-373, and Tersan OM (45% bis (dimethylthiocarbamoyl) disulfide, 10% hydroxymercurichlorophenol) were tested (Table 2).

Each material was tested at a single rate, with two applications at 3-day intervals (July 27,

---

[1]Contribution No. 260 of the Department of Botany and Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication as paper No. 2449 in the Journal Series.

[2]Associate Professor of Plant Pathology and Research Assistant, respectively.

Table 1. Effectiveness of nine fungicides in the control of the leaf lesion phase of melting-out of Kentucky bluegrass.

Material	Rate of application (ounces/1000 sq. ft.)	Date of application[a, b]	Disease rating[c]
Check	--	--	16.8
Dyrene	4	a	19.3
	4	a + b	64.3**
	8	a	32.0**
	8	a + b	81.0**
Upjohn GAB-5	2	a	18.3
	2	a + b	49.7**
	4	a	19.5
	4	a + b	58.2**
Acti-dione RZ	1.5	a	19.3
	1.5	a + b	44.2**
	3	a	28.3*
	3	a + b	59.8**
Hercules 3944-H	2	a	12.2
	2	a + b	19.8
	4	a	14.5
	4	a + b	28.2*
Hercules 4223-H	2	a	14.7
	2	a + b	22.0
	4	a	19.2
	4	a + b	31.0**
California Spray ML-372	4	a	16.8
	4	a + b	55.5**
	8	a	21.5
	8	a + b	64.5**
California Spray ML-373	4	a	22.5
	4	a + b	65.0**
	8	a	23.5
	8	a + b	76.3**
Orthocide 50W	6	a + b	31.8**
Kromad	4	a + b	39.4**

L.S.D. 5% = 10.8; 1% = 14.3

[a] = May 17, [b] = May 31, June 23.

[c] Percentage lesion-free leaves per sample.

Table 2. Effectiveness of eight fungicides in the control of Sclerotinia dollar spot of Seaside bentgrass.

Material	Rate of application (ounces/1000 sq. ft.)	Disease rating[a]
Check	4	77.5
Upjohn GAB-5	4	29.2**
Acti-dione RZ	1	39.2**
Hercules 3944-H	2	78.3
Hercules 4223-H	2	78.3
California Spray ML-372	4	25.8**
California Spray ML-373	4	16.2**
Dyrene	6	16.3**
Tersan OM	5	24.7**

L.S.D. 5% = 16.1; 1% = 21.5

[a] Percentage of plot surface blighted.

July 30), randomized in six replications (Table 2). Individual plots measured 4 feet x 20 feet. Materials were applied with a 10-gallon capacity, gasoline-powered sprayer, operating at 90 pounds' pressure. All treatments were made on the basis of dilution in 4 gallons of water per 1000 square feet of turfgrass.

The test area for the melting-out trials consisted of a second-year stand of Kentucky bluegrass. In September 1958 the plants in this area were inoculated in the following manner: Helminthosporium vagans was grown on autoclaved rye grains. After the fungus-permeated grains were air-dried, they were ground in a food mill. Distribution of the finely ground inoculum over the area was accomplished by means of a standard seeder-spreader. At the beginning of the trials disease severity was of a high order and uniform in incidence throughout the area.

The Sclerotinia dollar spot trial area was a second-year stand of Seaside bentgrass (Agrostis palustris). Inoculation with S. homoeocarpa was performed in April 1959, using the same rye-grain technique. Since the test area was managed at a 1-inch cutting height, the overall symptom pattern was more typical of that of the disease in home lawns, that is, large, blighted areas of turfgrass, rather than small, sharply outlined spots. At the time of the first fungicide applications, approximately 75% of the turfgrass surface was blighted due to Sclerotinia dollar spot.

Samples for the disease incidence ratings for the melting-out trials were taken 7 days after the time of the third fungicide application (Table 1). Rating was accomplished by employment of a slight variation of the leaf-sampling technique previously described (3). A 15-foot grid system of twine that provided 15 1-foot square sections arranged in a linear fashion was stretched over the center of each plot so as to orient its long axis with that of the area to be sampled. For each plot, four of the 1-foot sections were selected at random and 25 leaves taken from each. The individual leaves of each 25-leaf sample were then rated on the basis of presence or absence of lesions due to H. vagans. After this, the total 100-leaf rating for the plot was computed by averaging the four samples. Evaluation of level of disease control was based on percentage lesion-free leaves per sample. With this system, 600 leaves were rated per fungicidal treatment, with a total of 18,600 leaves for the overall test area.

Rating of the Sclerotinia dollar spot control trials was performed 5 days from the time of the second fungicide application. Disease incidence was scored on the basis of estimated percentage of blighted turfgrass area per plot. The final figure obtained for each formulation, then, was an average of the six replications.

All data were subjected to analysis of variance.

Control of melting-out of Kentucky bluegrass was accomplished with all of the materials tested. Single applications of Dyrene and Acti-dione RZ at the higher rates provided a satisfactory level of disease control throughout the 6-week test period (Table 1). This quality of turf produced with the Dyrene, Upjohn GAB-5, and California Spray ML-373 treatments was superior to that of the other formulations (including the two standard fungicide checks). Plant recovery in these plots was more rapid, and at the conclusion of the trials the stands were denser.

In the Sclerotinia dollar spot trials, all of the formulations tested, with the exception of Hercules 3944-H and 4223-H, provided satisfactory disease control (Table 2). The efficacy of the various entries is further demonstrated by the fact that such sharp reduction in disease incidence was effected in 8 days from the time of the first application.

The pentachloronitrobenzene component of Acti-dione RZ has been reported to be phytotoxic to Illahee fescue (Festuca rubra) (3). In the present trials, Acti-dione RZ was found to cause discoloration in both Kentucky bluegrass and Seaside bentgrass, with the latter species being slightly more prone to injury. This faint-yellow coloration was generally evident the day after application and persisted for 4 to 5 days.

DISCUSSION

In recent years effort has been directed toward the development of single-package formulations of fungicides effective in controlling a broad spectrum of turfgrass diseases. When as economical and effective as materials specific to individual disease problems, these formulations have many advantages, among which is the allowance for a wider latitude of error in diagnosis on the part of the user.

Realization of a broad spectrum of disease control through the use of a single formulation has been brought about primarily by the combination of materials known to be effective in the reduction of severity of diseases common to a given locality. All but three of the entries in the

present investigation were developed in this manner. Their effectiveness in controlling two of the more important turfgrass diseases in Pennsylvania makes them particularly valuable to the homeowner as well as the superintendent of highly specialized turfgrass areas.

The performance of Dyrene in the control of melting-out and Sclerotinia dollar spot indicates excellent possibilities for the material as a broad spectrum turfgrass fungicide in its own right, if not a principal ingredient in various combinations.

Literature Cited

1. COUCH, H. B. 1957. Melting-out of Kentucky bluegrass -- its cause and control. The Golf Course Reptr. 25(7): 5-7.
2. COUCH, H. B., and J. R. BLOOM. 1959. Turfgrass disease control. Bull. New York Turfgrass Assoc. 64: 248-250.
3. COUCH, H. B., and H. COLE, Jr. 1957. Chemical control of melting-out of Kentucky bluegrass. Plant Disease Reptr. 41: 205-208.
4. HOWARD, F. L., J. B. ROWELL, and H. L. KEIL. 1951. Fungus diseases of turfgrasses. Rhode Island University Agr. Exp. Sta. Bull. 308.
5. WADSWORTH, D. F., and H. C. YOUNG, Jr. 1959. Chemical control of diseases affecting turf of golf greens; progress report 1958. Oklahoma State University Agr. Exp. Sta. Processed Series P-319.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, THE PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA

THE USE OF THE ONION TEST AS A QUANTITATIVE METHOD FOR DETERMINING THE DISTRIBUTION OF EMULSIFIABLE DBCP IN SOIL

A. Rinkov, S. D. Van Gundy, R. L. Rackham, and M. J. Garber[1]

Abstract

The percentage reduction in growth of onion seedlings was highly significant in analysis for small quantities (2.5-10 ppm) of DBCP in sand and soil. The distribution of emulsifiable DBCP as measured by the onion test compared favorably with control of the citrus nematode. However, the onion test was faster and more reliable than nematode control when studying soil factors affecting the distribution of emulsifiable DBCP in soil. Of the soil factors tested, the clay content had the greatest influence on the distribution of emulsifiable DBCP in the six field soils tested.

ONION TEST

A rapid quantitative test for 1,2,dibromo-3-chloropropane (DBCP) was obtained by incorporating the Hoppe (6) "rolled towel" technique into the onion bromine test described by O'Bannon (7). Fifty cc of the soil sample was distributed in a rectangular area on a moist paper towel; then 15 Australian Brown Onion seeds (Allium cepa) were planted in a row on the soil and covered with another 50 cc of soil. The paper towel was rolled up and put into a quart Mason jar. Each soil sample was represented by three 100-cc sub-samples in each of which 15 onion seeds were planted. Three rolled towels were placed in a single jar. After 7 days of incubation at 70° F the "rolled towels" were removed, opened, and the onion seedlings measured from the "knee" of the cotyledon to the root-tip with a millimeter rule.

This method was standardized with known quantities of DBCP added in water in ppm per volume of sand or soil. The results of ten standardization tests are presented in Figures 1A, 1B, and 2A. The quadratic equation of the log-e dose was significant at the 1% level and was a straight line when plotted on semi-log paper except at the extreme concentrations (Fig. 2A). The standardization of 1 ppm DBCP fell below the theoretical response probably due to sorption of this small amount of chemical on the surface of the soil particles. Differences in growth response of the onion seedlings at concentrations above 10 ppm were difficult to measure accurately and usually inhibited germination of the seeds.

Soil from the Riverside I plot was subjected to the same standardization treatments to determine the differences between sand and soil. The onions were 10% less sensitive to DBCP in the clay soil. An interaction with the salt content of the soil was found when field soil taken from the first 6 to 8 inches inhibited germination and growth of the onion seeds in the standardization tests. This soil had provided good growth in earlier field tests. Germination and growth improved when soil was taken from the same area at the 1 to 2 foot depth. On checking, the surface soil was found extremely high in salts. It was concluded that this test could not be used in soils which were high in salts, unless sufficient leaching has occurred during the application of DBCP to remove the salts.

The moisture content of field soil should be about 75% of field capacity for best germination of the onion seeds. On a dry weight basis the moisture content of the sand standardization was 15%. Moisture contents of 5% or less and greater than 20% inhibited germination and growth. The "rolled towel" has the advantage of adjusting the soil moisture of field samples by using a dry paper towel when the soil is wet or a damp paper towel when the soil is dry. Since the growth of the check onion seedlings varied from soil to soil, the percentage reduction in the treated onion seedlings was used for comparison of field results and for estimating ppm of DBCP in the field samples. Preliminary tests on the influence of moisture in sand showed that the percentage reduction remained nearly constant even though the growth of the seedlings varied considerably.

FIELD APPLICATIONS OF EMULSIFIABLE DBCP

The distribution of DBCP as measured by the onion test and control of the citrus nematode Tylenchulus semipenetrans was tested in six citrus soils. Metal basins described by Baines

[1]Laboratory Technician, Assistant Nematologist, Laboratory Technician, Assistant Biometrician, University of California, Citrus Experiment Station, Riverside, California.

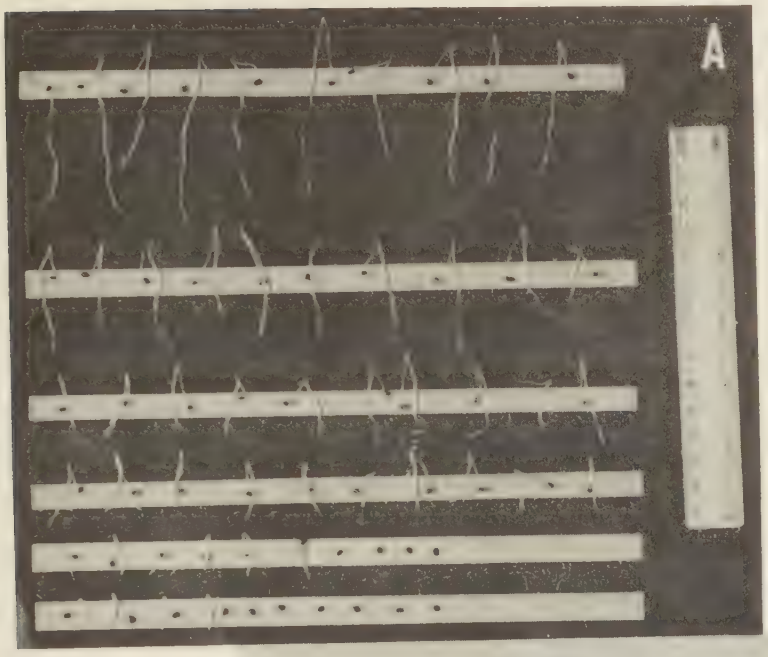


FIGURE 1. A -- The influence of DBCP on the germination and growth of onion seedlings at (top to bottom) check, 2.5, 5, 10, 50, 100 ppm. B -- Materials used in standardization technique.

et al. (2) were used as containers for the application of the chemical in irrigation water. The fumigant used was Shell Nemagon Emulsifiable 50% by volume. The rates reported (2.5 and 5 gallons/acre in 6 acre-inches of irrigation water) are gallons of active material per acre. Each treatment was replicated three times.

The distribution of DBCP in the field soil was measured by the onion test 7 days after treatment. This period was usually sufficient to allow the soil to dry enough for sampling. Since DBCP has a high boiling point and a low vapor pressure, little movement was expected to have occurred during this period due to diffusion. Each plot was sampled at 6-inch increments to a depth of 24 inches with a 3-inch soil auger. Three "rolled towels" per treatment were made at the sample site so that as little DBCP as possible was lost during the sampling operation.

Soil samples for nematode counts were taken from each plot at 1-foot increments to a depth of 3 to 6 feet with a 1-inch soil tube approximately 3 months after application. Each sample was a composite of three 1-inch soil cores per replicate. The nematodes were extracted as outlined by Baines et al. (1). The soil temperature in the first foot and percentage moisture in

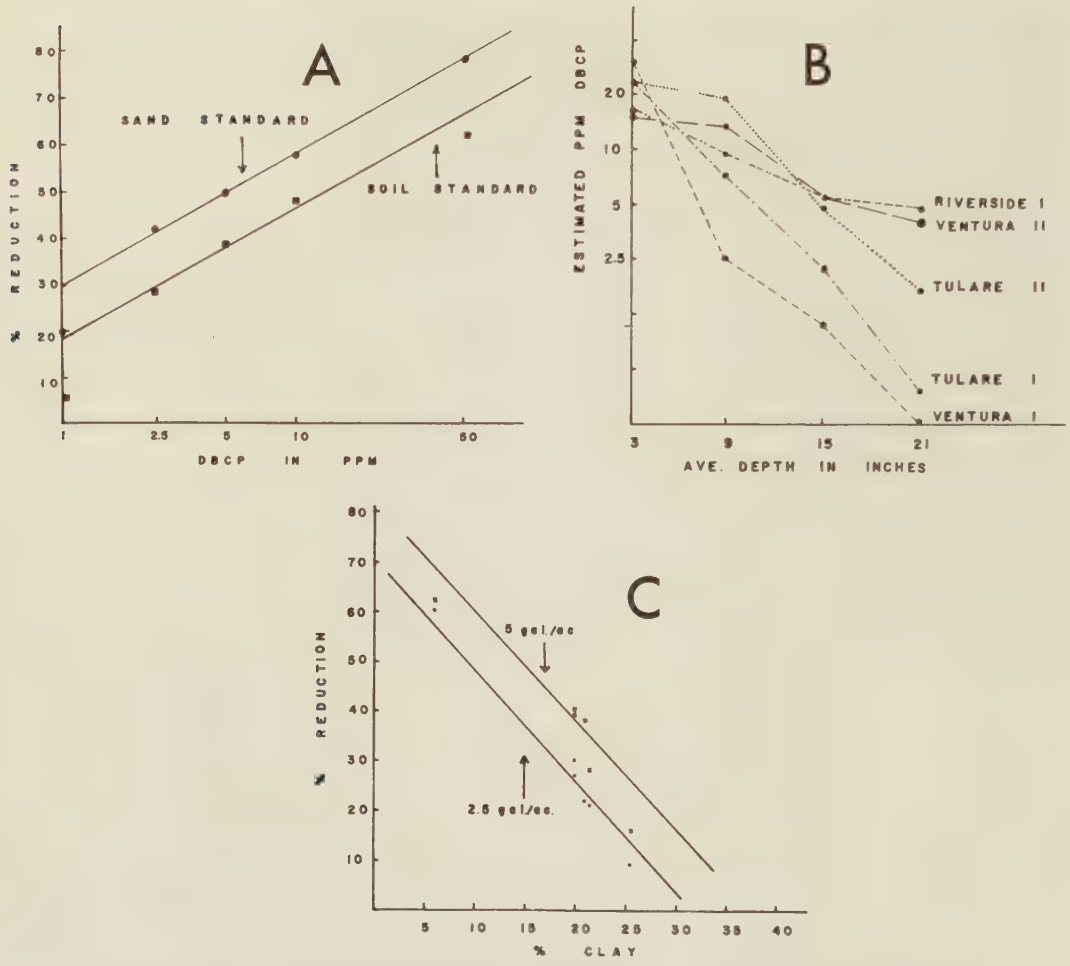


FIGURE 2. A -- The standardization of DBCP in ppm per volume of soil based on percentage reduction of growth of onion seedlings. B -- Distribution in ppm of 5 gallons/acre of DBCP in 6 inches of water in five field plots based on percentage reduction of growth of onion seedlings in the soil standard. C -- Influence of percentage clay of six field soils on the distribution of 2.5 (.) and 5 gallons/acre (x) DBCP at 6 to 12 inches and 12 to 18 inches respectively as measured by the percentage reduction of growth of onion seedlings.

the first 2 feet were measured at the time of application. The soils from the first 2 feet of each plot were also analyzed for particle size using the hydrometer method as described by Day (5), organic matter content as described by Schollenberger (8), and cation exchange capacity using the sodium method outlined by Bower et al. (4).

Distribution of Emulsifiable DBCP in Different Soils

Growth of the onion seedlings in soil samples obtained at the different depths in six soils treated with several dosage levels of DBCP is illustrated in the three dimensional graphs in Figure 3. The sources of variability and statistical significance are presented in Table 1.

The most uniform distribution of DBCP was in Riverside II plot which was composed of 80% sand. In comparing the reduction in growth of onion seedlings from the Riverside II plot with the reduction in growth in the sand standard, it appeared that the entire soil mass through 2 feet contained approximately 10 ppm which was the theoretical amount 5 gallons/acre would provide if mixed uniformly. The soil standard more nearly represented the soil texture of the other five plots and could be used to account for approximately 90% of DBCP added in each case (Fig. 2B). The ability of the onion test to account for nearly all the material added illustrates the value of the test.

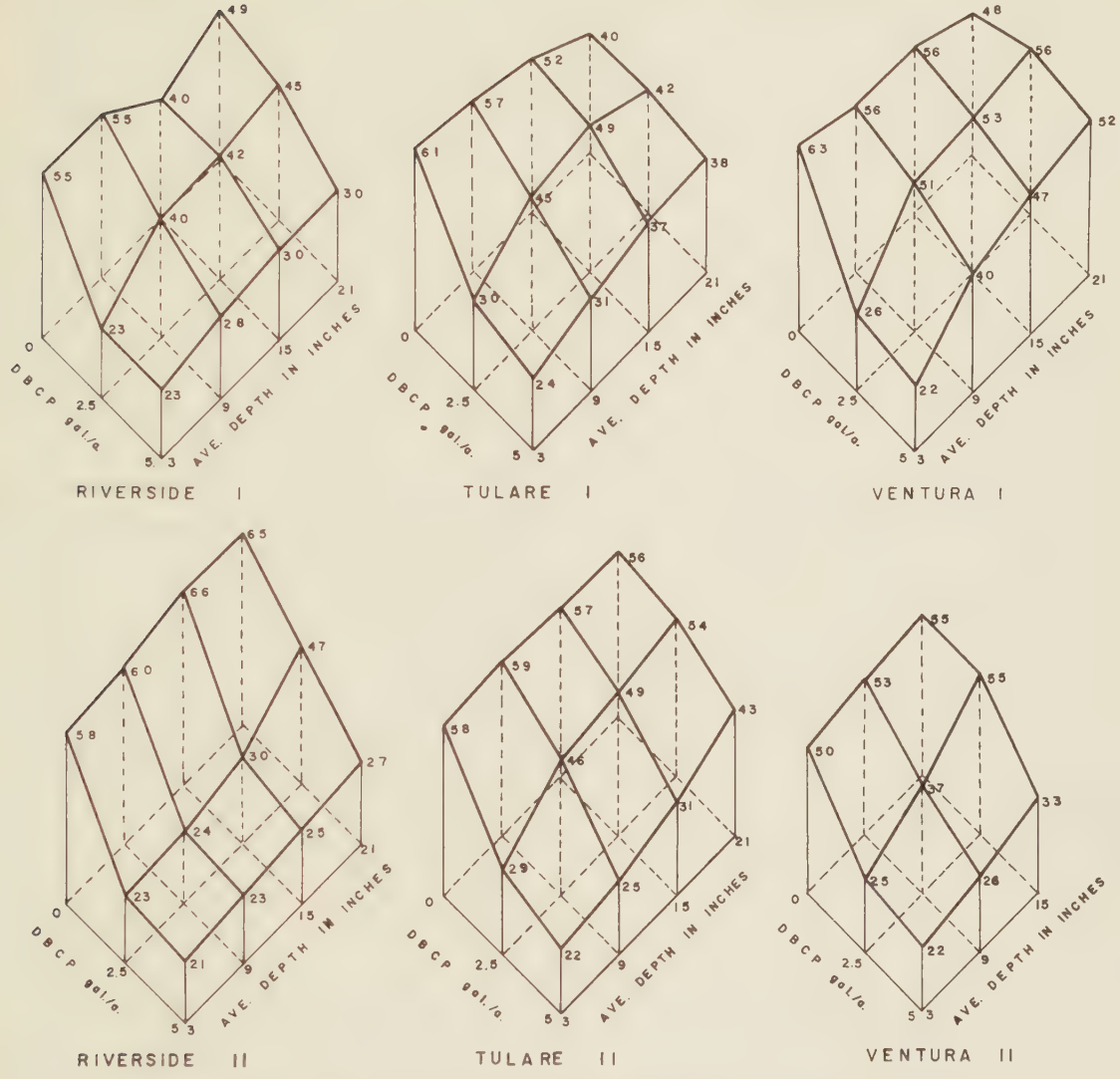


FIGURE 3. The effect of non-treated and DBCP treated soil taken at different depths on the growth of onion seedlings in millimeters.

The distribution of 5 gallons/acre in a soil such as Riverside I was not as good as 2.5 gallons /acre in a soil such as Riverside II. However, the distribution of 2.5 gallons/acre in the Riverside I soil was just as effective as 5 gallons/acre in the Ventura I soil. With the amount of water constant, it appeared that distribution within the various soils was dependent upon some soil factor or factors.

The soils in Table 2 are listed in order from the greatest to least concentration of DBCP at the 21-inch depth as measured by the onion test. The order of the soils then appeared to be associated with the amount of clay present. The cation exchange capacity of the soils tested was also associated with increasing clay content and therefore may be related to the pattern of distribution of DBCP at the higher levels. The soil from the first foot of the Tulare I plot had the same amount of clay as the Tulare II, Ventura II and Riverside I plots. Their pattern of distribution was similar. However, the second foot of soil of Tulare I was considerably higher in clay and the distribution closely paralleled that of Ventura I. The soils from Riverside I and Ventura II are nearly the same so far as soil texture is concerned but differ nearly twofold in their cation exchange capacity and percentage organic matter; yet the pattern of distribution of DBCP was similar. The percentage clay of the soils tested shows a correlation with the distribution of emulsifiable DBCP in the soil as shown in Figure 2C. This may be explained in the

Table 1. Sources of variability and statistical significance of the growth of onion seedlings when grown in non-treated and DBCP treated soil taken from different depths.

	Riverside I	Riverside II	Tulare I	Tulare II	Ventura I	Ventura II
Treatment						
linear	***[a]	***	***	***	**	***
quadratic	NS	***	NS	NS	NS	NS
Depth						
linear	NS	***	NS	***	***	***
quadratic	NS	NS	**	NS	**	NS
cubic	NS	NS	NS	NS	NS	NS
Treatment x depth						
linear x linear	*	NS	***	**	***	NS
linear x quadratic	NS	NS	*	NS	NS	NS
linear x cubic	NS	NS	NS	NS	NS	NS
quadratic x linear	**	**	NS	*	**	*
quadratic x quadratic	NS	*	*	NS	NS	NS
quadratic x cubic	NS	NS	NS	NS	NS	NS

[a]NS: not statistically significant.

*, **, ***: statistically significant at the 0.05, 0.01, and 0.001 levels of probability, respectively.

Table 2. The classification and properties of six soils treated with DBCP.

General classification of plots	Depth (in feet)	Mechanical analysis (%) sand	silt	clay	Temperature at 8 inches (°C)	Average moisture content	Average cation exchange capacity	Average % organic matter
Riverside II	0-1	81	12	7	22-23	6.0	2.75	0.2
(loamy sand)	1-2	80	13	7				
Riverside I	0-1	56	24	20	26-28	6.0	9.35	1.1
(sandy loam)	1-2	55	25	20				
Ventura II	0-1	49	31	20	21-22	9.5	14.85	2.1
(loam)	1-2	44	35	21				
Tulare II	0-1	42	36	22	30-32	8.0	19.80	1.4
(loam)	1-2	42	36	22				
Tulare I	0-1	45	32	23	25-32	11.8	16.83	1.2
(loam)	1-2	39	29	32				
Ventura I	0-1	33	41	26	22-23	9.3	26.40	2.7
(clay loam)	1-2	32	41	27				

Table 3. Control of citrus nematode 3 months after treatment with 2.5 and 5.0 gallons/acre of DBCP applied in 6 inches of water in basins.

Sample depth (in feet)	Average number of citrus larvae per 50 cc of soil: Tulare I Check	2.5	5.0	Tulare II Check	2.5	5.0	Ventura II Check	2.5	5.0
0-1	300	0	0	62	0	0	331	162	0
1-2	202	26	3	267	30	0	75	153	0
2-3	55	435	39	175	44	445	a		

	Ventura I			Riverside I			Riverside II		
0-1	1525	6	0	2242	0	0	228	0	0
1-2	1473	2196	468	2807	0	0	74	0	0
2-3	435	148	346	2335	41	0	91	0	0
3-4	318	786	842	1302	321	2	1	0	0
4-5							0	0	0

[a]Samples not obtained due to rocky subsoil.

following manner. Clay soils such as those used here have approximately 20 times as much surface area as the sandy soil. The fine clay particles may sorb or filter out the DBCP. Although the waterholding capacity of these soils was not measured, the soils expected to have a considerably higher moisture holding capacity should decrease the depth of penetration of a given amount of water. This in turn should reduce the distribution of DBCP at the lower depths. Also, any amount of compaction of the clay soils would decrease the number of interconnecting pores present for the rapid movement of the water and fumigant. In all cases the water appeared to move through the soil farther than did the DBCP.

Diffusion of DBCP as Measured by Nematode Control

The results on control of the citrus nematode larvae 3 months after treatment are presented in Table 3. Nematode control in the Ventura I and Ventura II soils was the poorest and differed from the other soils by their higher percentage of organic matter. There was no indication in these two soils that the DBCP had diffused through the soil any farther than it had been distributed 7 days after treatment. In fact, at the lower dosages it failed to completely control the nematodes in the soil it had penetrated. For example, the distribution of 2.5 gallons/acre on the Riverside I was only slightly better than the 5 gallons/acre on the Ventura I plot, yet the control with 2.5 gallons/acre on the Riverside I plot was considerably better. The clay content differed by only 6 to 7%. However, the percentage organic matter of the Ventura I soil was 2 1/2 times greater than that of the Riverside I soil. Essentially the same is true in the case of the Riverside I and Ventura II plots. In comparing the 2.5 gallons/acre rates, the distribution pattern was similar yet they differed greatly in nematode kill. The soil texture of these two soils was similar; however, the Ventura II soil contained twice the amount of organic matter.

The nematode control on the Tulare I and Tulare II plots was not so good as that obtained on the Riverside I plot. This may have been due to the somewhat higher silt, clay, and organic matter content. Baines et al. (3) reported that the percentage clay and silt in the soil reduced the diffusion of DBCP when injected to a depth of 8 inches.

The distribution of DBCP as measured by the onion test compared favorably with nematode control. However, nematode control was influenced by soil factors already known to adversely affect the diffusion of DBCP and similar fumigants. It appeared that the onion test was a faster and more reliable method than the nematode control for studying factors affecting the distribution of emulsifiable DBCP in the soil at the time of application.

Literature Cited

1. BAINES, R. C., F. J. FOOTE, L. H. STOLZY, R. H. SMALL, and M. J. GARBER. 1959. Factors influencing control of the citrus nematode in the field with D-D. Hilgardia 29: 359-380.
2. BAINES, R. C., R. H. SMALL, T. A. DeWOLFE, J. P. MARTIN, and L. H. STOLZY. 1957. Control of the citrus nematode and Phytophthora spp. by Vapam. Plant Disease Reptr. 41: 405-414.
3. BAINES, R. C., L. H. STOLZY, O. C. TAYLOR, R. H. SMALL, and G. E. GOODALL. 1958. Nematode control on bearing trees. California Citro. 43: 328-329.
4. BOWER, C. A., R. F. REITEMEIER, and M. FIREMAN. 1952. Exchangeable cation analysis of saline and alkali soils. Soil Science 73: 251- 261.
5. DAY, P. R. 1956. Report of the Committee on Physical Analyses. Soil Sci. Soc. of Am. Proc. 20: 167-169.
6. HOPPE, P. E. 1955. Rolled towel paper method. Wisconsin Agr. Exp. Sta. Bull. 507.
7. O'BANNON, JOHN H. 1958. Application of emulsifiable dibromochloropropane in irrigation water as a preplanting soil treatment. Plant Disease Reptr. 42: 857-860.
8. SCHOLLENBERGER, C. J. 1927. A rapid approximate method for determining soil organic matter. Soil Science 24: 65-68.

DEPARTMENT OF PLANT NEMATOLOGY AND BIOMETRICAL LABORATORY,
UNIVERSITY OF CALIFORNIA, CITRUS EXPERIMENT STATION, RIVERSIDE, CALIFORNIA

SPRING DEAD SPOT OF BERMUDAGRASS

D. F. Wadsworth and H. C. Young, Jr.

Abstract

A disease of bermudagrass turf called spring dead spot has become prevalent in Oklahoma. During the past 3 years bermudagrass has suffered extensive damage which is characterized by dead areas of turf that are apparent only after the grass begins to grow in the spring. The cause of the disease is not known, but it appears to be due to fungi which attack the root system while the grass is dormant.

A disease, which is now called spring dead spot, was observed in a bermudagrass (Cynodon dactylon) lawn at Stillwater, Oklahoma during the spring of 1954. Since that time, this disease has been found throughout much of the State on lawns, golf courses and many other public and private turf areas. The prevalence and severity of spring dead spot has steadily increased and during the past 3 years has become the most important disease of bermudagrass in Oklahoma.

Conversation and correspondence with golf course superintendents and other turf grass area managers has led to the conclusion that this disease may have been present, at least locally, for many years. The only concrete information, however, came from Mr. Bob Dunning of Tulsa, Oklahoma, who believes in the light of present investigations that he observed spring dead spot as early as 1936.

Reports which indicate the distribution of the disease are rather vague. It was reported from Kansas in 1959[1], and was observed by the senior author in the turf plots at the University of Nebraska, Lincoln, Nebraska, the same year. Following a discussion of the disease at the Annual Conference of the Oklahoma Turfgrass Association in December 1959, various individuals reported that this, or a similar disease, had been seen in Pennsylvania, Missouri, and Arkansas.

SYMPTOMATOLOGY

When bermudagrass begins to grow in the early spring well defined, circular, dead spots may be present (Fig. 1). Individual spots vary in size from a few inches to 3 or 4 feet in diameter. The margins are usually even but may become irregular when spots have coalesced to form large, dead areas several feet across. Foliage of the dead grass is a bleached straw color, while the stolons and roots are black and rotted. These plant parts characteristically appear

FIGURE 1. Spring dead spot of common bermudagrass on a golf course fairway, Tulsa, Oklahoma, 1958.

[1]King, Claude L. 1959. A plant disease newsletter directed to County Agricultural Agents. Kansas State University, Manhattan, Kansas. (Mimeographed)

to have been dead for some time, and there is no obvious indication at any time during the year that the causal agent of the disease is active. In other words, there are no obvious preliminary symptoms. The damage appears to occur while the grass is dormant; therefore, the appearance or spread of the disease can be observed only each spring when grass resumes growth.

With few exceptions all of the grass in the affected spots is dead. Occasionally a tuft of grass may survive in the larger spots and, where this occurs, the affected area is doughnut shaped (Fig. 2). The dead area of such spots may fill from the inside to the outside in 3 or 4

FIGURE 2. A doughnut-shaped effect produced by the recovery of bermudagrass in an area affected by spring dead spot.

years. Usually, however, the larger dead spots will remain void of bermudagrass for a number of years and other grasses and weeds become well established in the affected areas during this time. The presence of this disease often can be detected by this particular pattern of plant invasion.

There is a tendency for the surrounding grass to fill in the smaller spots when conditions are favorable. This occurs if the stolons bridge the dead spots and become rooted on the far side. Stolons that fail to bridge eventually die, as the new, small roots rot away.

The disease has not been associated with any particular type of soil or topography.

HOST RANGE

Spring dead spot has been observed only on bermudagrass. It is known to occur on the varieties African, U-3, Common, Tiffine, and Tifgreen; however, the most extensive damage has occurred on U-3. A number of other bermudagrass varieties have been introduced in recent years but their reaction to this disease has not been determined.

Spring dead spot on bermudagrass has been observed only under conditions of management which produce a high quality turf and not under conditions with a pasture grass type of management.

ETIOLOGY

Bermudagrass sometimes suffers from winter injury, snow mold, and insects, and these types of damage are commonly mistaken for spring dead spot. The true cause of the disease now known as spring dead spot, however, is not known. Fungi are found consistently associated with the disease and on extremely rare occasions white grubs and/or plant parasitic nematodes are present in small numbers. Isolations from diseased tissues yield many fungi; however, only certain unidentified species of Helminthosporium are consistently obtained.

African, U-3, Sunturf and Common varieties of bermudagrass have been inoculated with eight different isolates of Helminthosporium; some of the inoculations have been in the greenhouse and some in the field. No symptoms of spring dead spot developed in any of these tests.

Bermudagrass taken from the periphery of dead spots and transplanted by various methods into flats of sterilized or screened soil and subjected to intervals of growth and dormancy over a 3-year period failed to develop symptoms of the disease.

Spring dead spot occurs most frequently in areas where high quality turf has been established; consequently, it was thought that certain fertilizer practices may influence the develop-

ment of the disease. However, after comparing the fungicidal-fertilizer, calcium cyanamide, and ammonium nitrate for 3 years, there appears to be no difference in their effect on disease development.

Further studies on etiology and control of spring dead spot area are in progress.

DISCUSSION

It seems likely that the recent importance of spring dead spot is due to greater prevalence than in previous years. The increase in disease prevalence may be the result of greater use of bermudagrass turf for home lawns and public areas, and since spring dead spot is found only in well cared for turf, it is possible that the disease will continue to increase in prevalence and importance.

The cause of spring dead spot is not yet known. However, observation of the disease since 1954 leads to the conclusion that it is due primarily to root-rotting fungi. The damage appears to occur during the winter season while the grass is dormant, which might suggest that the causal agent involved is a cool weather pathogen. This suggestion may be misleading, however, since there are frequent warm periods of short duration throughout the winter, at least, in the southern and southwestern areas of the United States.

Many fungi have been obtained by isolating from the rotted root systems and stolons but the only fungi found consistently were Helminthosporium spp. Several species of this genus have been isolated and any one or all may have been involved in the disease. If one or more of these species of Helminthosporium were involved, however, the conditions favorable for infection and/or disease development were not easily reproduced.

White grub worms and plant parasitic nematodes have been found associated with spring dead spot also but not with sufficient regularity to be considered as primary causal agents. When either or both of these agents were associated with the disease they probably only enhanced the damage already done.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, OKLAHOMA AGRICULTURAL EXPERIMENT STATION, OKLAHOMA STATE UNIVERSITY, STILLWATER

STUDIES ON THE PHYTOPHTHORA ROT OF SUGARCANE SEED PIECES IN LOUISIANA

T. van der Zwet, I. L. Forbes and R. J. Steib[1]

INTRODUCTION

The rotting and deterioration of sugarcane seed pieces is a problem present in almost every sugarcane-growing country in the world. In the tropical areas pineapple disease, caused by Ceratostomella paradoxa, is considered the most important seed piece rot, while in the subtropical areas red rot, caused by Physalospora tucumanensis, is the one causing most damage in this respect. Since the plant cane in Louisiana lies dormant in the soil for about 6 months after it has been planted in September and passes through a period of unfavorable climatic conditions during this time, the vitality of the cane is easily lowered and the seed pieces become more susceptible to the attack of certain soil microorganisms. Sometimes this winter period has been unusually wet and accompanied by extra low temperatures. This condition was observed during the winter and early spring seasons of 1947 and 1948, and the variety C.P. 36-13 failed to germinate in certain areas of the sugarcane belt. This variety had just been released for commercial use and was considered resistant to mosaic and moderately so to root rot (1). Since it was also considered highly resistant to red rot it was hard to believe that red rot could be the cause of the failure.

In the early stages of infection, Phytophthora rot can easily be distinguished from red rot by the internal symptoms of the diseased seed pieces. Red rot is characterized by a deep red discoloration of the internal tissue and the presence of typical transverse white blotches in the internodal region, while Phytophthora rot shows a typical orange-pink discoloration of the tissue, without the presence of any white spot (Fig. 1). In the more advanced stage of Phytophthora infection, the tissue turns reddish and purplish brown and often has a distinct ether-like odor.

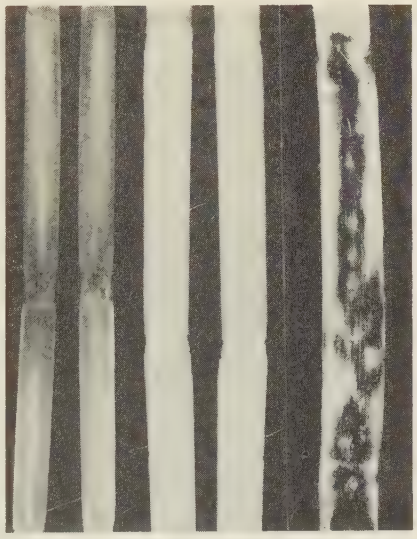


FIGURE 1. Typical disease symptoms of Phytophthora rot (left) and red rot (right). Healthy cane in center.

Chilton and Steib (2) gave the first account of this rather unique Phytophthora rot in Louisiana in 1948 and until today the disease has not been reported elsewhere. Later these investigators reported that of 2017 nodes and internodes plated 51.5% gave Phytophthora isolates, which were divided into three distinct cultural types (6). When these Phytophthora strains were examined one was found to resemble Pythium in its morphological characteristics; the third was never identified, due to the complete absence of any fruiting structures, and was therefore tentatively called the "sterile" mycelium isolate. This last isolate, however, was found to be the

[1] Respectively, Plant Pathologist, United States Department of Agriculture, Field Laboratory for Tung investigations, Bogalusa, Louisiana and Professor and Associate Professor, Department of Plant Pathology, Louisiana State University, Baton Rouge, Louisiana.

Two were identified as P. erythroseptica Pethyb. and

most pathogenic one in seed piece inoculation experiments. Sanchez-Navarrete (4) and Singh (5) made several attempts to induce fruiting in the "sterile" Phytophthora isolate, but were rather unsuccessful. The latter did, however, observe some sporangia and oospores on sugarcane roots.

The present study was undertaken 1) to try to induce fruiting and to attempt identification of the most pathogenic Phytophthora isolate, 2) to study the basic method of seed piece infection and the rate of spread of the pathogen inside the seed cane, and 3) to determine the relative susceptibility of several past and present commercial sugarcane varieties as well as some yet unreleased experimental test field varieties to the disease under field conditions.

THE CAUSAL ORGANISMS

In the laboratory studies the "sterile" Phytophthora isolate was grown on sterilized oats in large flasks, which inoculum was intended for use in the field tests, to be planted with the seed pieces in the furrow. The flasks were stored in a constant temperature room at 70° F, and it was on these grains that fruiting bodies of the isolate were discovered. The fungus produced oogonia and antheridia in a manner typical for the genus Phytophthora and oospores were observed in abundance. When these grains with the fungal growth were placed in water, sporangia and zoospores were produced profusely. After comparative studies with seemingly related species of Phytophthora, in respect to morphology, growth-temperature relations and pathogenicity, this pathogenic isolate was identified as being similar to Phytophthora megasperma Drechs. Phytophthora seed piece rot of sugarcane is therefore now known to be caused by two Phytophthora species, namely the earlier reported and slightly pathogenic P. erythroseptica and the more virulent, recently identified P. megasperma. Some of the typical morphological characteristics of this sugarcane isolate were described by the senior author in an earlier publication (7).

SEED PIECE INFECTION

In order to study the basic method of infection of seed pieces by Phytophthora, an experiment was set up in which two-eyed seed pieces of the varieties N.Co. 310 and C.P. 36-13 were planted in sterilized soil, artificially infested with P. megasperma. These seed pieces were dug and examined at 2, 3, 5, 7, and 9 weeks after planting, and it was evident in the first examination that many of the root primordia and buds were red discolored, swollen, and infected with Phytophthora (Fig. 2). At the end of 9 weeks the variety N.Co. 310 had 55.5% of

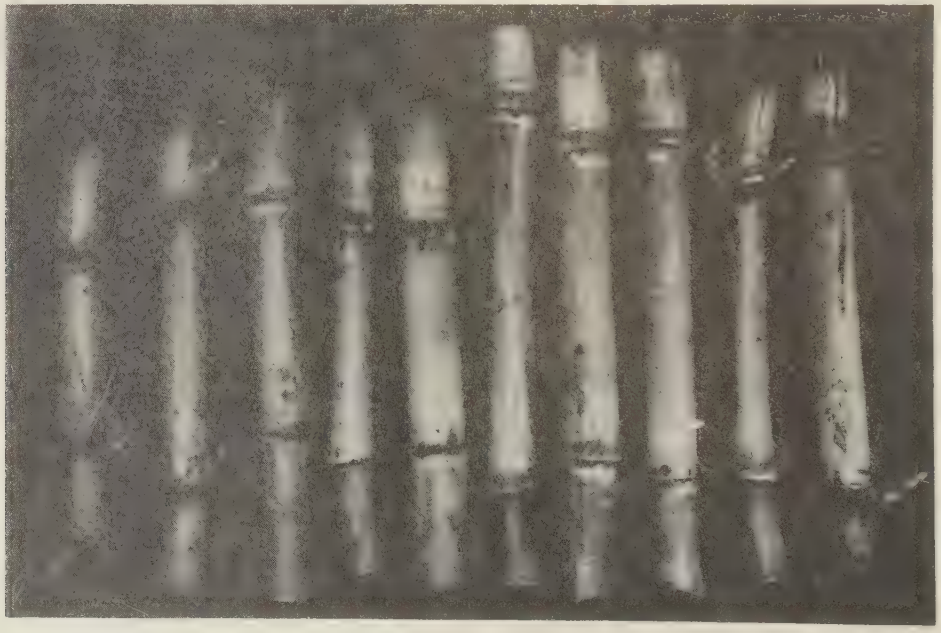


FIGURE 2. External nodal infection of C.P. 36-13 (left) and N.Co. 310 (right) by P. megasperma. Checks at extreme left and right.

its root primordia and 70.0% of its buds infected, while for the variety C.P. 36-13 the percentages were 67.9 and 80.0 respectively. The most interesting phenomenon observed in this infection experiment was that the root primordia and buds of the seed pieces planted in infested soil, which had escaped infection by the pathogen, had not germinated at all, while those in the checks started germinating 3 weeks after planting. After 7 weeks both varieties planted in non-infested soil had 70.0% of the buds germinated, while their root systems were quite abundant (Fig. 2). From additional preliminary laboratory studies, it is suggested that a toxin is possibly produced by the fungus, which may inhibit the germination process.

In order to study the rate of spread of Phytophthora in artificially infected seed cane, two-eyed seed pieces of the same two varieties were inoculated with P. megasperma by cutting a hole in each internode and placing oat grains with the fungus in each hole. The seed pieces were planted in sterilized soil and were dug for examination at 10, 15, 20, 30, and 50 days after inoculation. When the canes were examined, the typical pink discoloration and water-soaked appearance of the tissue was observed 10 days after inoculation; later the color turned purplish, giving off an ether-like odor. The diseased portion of the internode reached the adjacent nodal regions 20 days after inoculation, while after 50 days the internode had almost entirely deteriorated. Phytophthora spread more rapidly in the variety N.Co. 310 than in C.P. 36-13.

VARIETAL SUSCEPTIBILITY

An extensive varietal field test was carried out during the winter and spring seasons of 1957-1958 and 1958-1959, in which many past and present commercial sugarcane varieties and some unreleased test field varieties were tested under field conditions in regard to their susceptibility to Phytophthora seed piece rot. The winter and spring months of 1957-1958 were very wet and accompanied by low temperatures, which made conditions extremely favorable for the development of seed piece rot. The following winter and spring, however, were very mild with below normal rainfall. The canes, which were planted in October, were tied in bundles of five stalks and placed in the furrow to which many oats with the fungus had been added previously. The seed pieces were dug and examined at monthly intervals during February, March and April of each spring season, in order to check them for progressive infection and spread of the pathogen. After the canes were dug and freed of excess soil, each stalk was thoroughly washed and split lengthwise to be examined internally, while a count was made of the number of diseased nodes and internodes. All tissues suspected of being infected by Phytophthora were taken into the laboratory to be plated out on oatmeal agar plates according to standard procedures. Two separate plantings of the varieties were made in the fall of 1958, one of which was used to make a stand count during the following spring. From the data collected during this 2-year testing program, including those on germination and growth of the varieties during the spring and summer of 1959, the following breakdown of the varieties is suggested:

Susceptible	Intermediate	Resistant
C.P. 28-19	C.P 807	C.P. 29-116
C.P. 34-120	C.P. 36-105	C.P. 43-47
C.P. 36-13	C.P. 44-155	C.P. 44-101
C.P. 44-154	C.P. 52-68	C.P. 47-193
C.P. 53-1		C.P. 48-103
C.P. 53-15	P.O.J. 36, 213, 234	C.P. 48-103
C.P. 53-22	Co. 281, 290	C.P. 51-21
C.P. 55-30		N.Co. 310

Of the 24 sugarcane varieties listed, C.P. 44-155 and C.P. 53-15 were found to be the most susceptible to the seed piece rot in respect to severity of the initial nodal infection and rate of spread of the pathogen in the seed cane; C.P. 48-103 can be considered the most resistant variety. Regarding the presently grown commercial varieties in Louisiana, all are quite or at least partially resistant to the seed piece rot. The variety C.P. 52-68 was found to be quite susceptible during the 1957-1958 testing season.

Since the P.O.J. and Co. varieties could not be tested in the stand count experiment during 1958-1959, they are listed together in the intermediate column. The varieties P.O.J. 36, P.O.J. 213 and Co. 290, however, were found to be very susceptible during the winter 1957-1958, which was most favorable for the occurrence of Phytophthora seed piece rot.

DISCUSSION

The present study has definitely proved that cold wet weather is essential for severe development of Phytophthora seed piece rot. The date of planting seed cane can also be of importance. In a limited survey conducted during the spring of 1958 it was found that seed cane planted from late September until November gave higher percentages of Phytophthora cultures from diseased internodes than those planted in August or early September. In the present study it was also found in some cases, however, that young "stubble" produced by seed cane of the variety C.P. 36-13, planted in August of 1957, was water-soaked in appearance and had almost completely deteriorated. A definite line (Fig. 3, see arrow) could be observed between the diseased underground portion of the "stubble" and the yet healthy part above ground. Since cultures of P. megasperma were consistently isolated from the less deteriorated parts of these false stubbles, this seems to indicate that Phytophthora rot may be of importance in the stand failures which occur in summer planted seed cane following a winter of low temperatures and high rainfall. Hebert and Matherne (3) pointed out some advantages and disadvantages of the date of planting seed pieces in respect to yields of cane and sugar. They also stated that the depth of placement of the seed cane with respect to the water furrows should be varied from relatively deep in the better-drained, light soils to relatively shallow placement in the heavier, poorly-drained soils. These facts are of indirect importance in Phytophthora seed piece rot. Many investigators have suggested that red rot was responsible for the failure of varieties P.O.J. 213 and Co. 290 in Louisiana during the early 1930's and 1940's respectively. In a comparison of the weather conditions of these two periods, the fall of 1929 was one of the wettest falls on record, while in the winter following the number of hours below freezing was greater than for previous or later winters. Almost similar conditions prevailed during the winter and spring seasons of 1939-1940.

FIGURE 3. False stubble of the variety C.P. 36-13 infected with Phytophthora.

In the present field testing program during the cold and wet year 1957-1958 the varieties P.O.J. 213 and Co. 290 were very susceptible to Phytophthora rot, while during the following dry and mild year they were much less susceptible to the disease. Since the years 1929-1930 and to a lesser extent 1939-1940 were rather cold and very wet, and because of the fact that a relatively high number of Phytophthora cultures were recovered from both these varieties during the spring of 1958, Phytophthora seed piece rot might have played an important role in the stand failures of the varieties P.O.J. 213 and Co. 290 in those past periods.

In regard to the disease epidemics in the variety C.P. 36-13 in certain areas of the Louisiana sugarcane belt in the spring of 1947 and 1948, which resulted in the first announcement of Phytophthora seed piece rot, results of the present investigation indicate this failure was due

entirely to that seed piece rot. The average temperature during the winter months of 1946-1947 and the following year were rather low, while both seasons had excessive amounts of rainfall. Average temperatures were near a record low for both February and March of 1948. These weather data, together with the high number of Phytophthora cultures and few red rot cultures isolated in the present study from seed pieces of this variety during the spring of 1958, indicate that Phytophthora seed piece rot was entirely responsible for the epidemic in this variety during those 2 years.

An interesting observation made in the present investigation was the fact that the seed pieces became infected more easily through the nodal regions than through the cut ends of the canes, as would ordinarily be expected and which had been found to be the case in pineapple disease and black rot of sugarcane. The occurrence of this nodal infection at the yet ungerminated root primordia and buds is therefore an important factor in the development of Phytophthora rot, especially since in Louisiana whole stalks are used for seed cane, in contrast to the tropical cane-growing countries. When cane is planted in the fall of the year and cold weather sets in early, accompanied by heavy precipitation which will keep the soil saturated, Phytophthora seed piece rot can become very important, especially to the more susceptible varieties. However, a cool wet spring also is important in the inducement of Phytophthora rot. A cool spring prevents the cane from germinating, but not the disease from occurring.

Literature Cited

1. BRANDES, E. W., W. G. TAGGART, and G. L. BILLEAUD. 1946. Release of new variety seed cane C.P. 36-13. Sugar Bull. 24: 167.
2. CHILTON, S. J. P., and R. J. STEIB. 1948. A Phytophthora rot of seed cane. Louisiana Agr. Exp. Sta. Ann. Rept. 1947-48: 95-96.
3. HEBERT, L. P., and R. J. MATHERNE. 1959. Effect of depth of placement of seed cane and date of planting on yields of cane and sugar from C.P. 36-105 and C.P. 44-101 at Houma, Louisiana. Sugar Bull. 37: 260-266.
4. SANCHEZ-NAVARRETE, F. 1950. Factors relative to Phytophthora rot of sugarcane. Thesis, Louisiana State University.
5. SINGH, K. 1955. Phytophthora rot of sugarcane. Dissertation, Louisiana State University.
6. STEIB, R. J., and S. J. P. CHILTON. 1950. The Phytophthora rot of sugarcane seed pieces in Louisiana. Intern. Soc. Sugar Cane Tech., Proc. 7th Cong. 524-528.
7. van der ZWET, T. 1959. Studies on a Phytophthora isolate causing seed piece rot of sugarcane in Louisiana. (Abst.) Phytopathology 49: 320.

LOUISIANA STATE UNIVERSITY, BATON ROUGE, LOUISIANA

USE OF HERBICIDES TO BREAK THE LIFE CYCLE OF THE BENTGRASS NEMATODE, ANGUINA AGROSTIS (STEINBUCK 1799) FILIPJEV 1936[1]

Walter J. Apt, H. M. Austenson, and Wilbur D. Courtney[2]

Abstract

The herbicides amitrol, dalapon, and maleic hydrazide show promise for controlling the bentgrass nematode, Anguina agrostis (Steinbuck 1799) Filipjev 1936. At appropriate rates they can suppress heading of bentgrass for a year, thereby breaking the life cycle of the nematode. In one experiment gall production was reduced from 70% to 2%. One seed crop was sacrificed by the treatments, but the succeeding crop was sufficiently increased to compensate this loss. These chemical control methods are more effective and cheaper than summer-fallowing or other mechanical methods.

INTRODUCTION

The coastal area of southwestern Washington and northwestern Oregon has long been one of the major seed production regions for Colonial bentgrass, Agrostis tenuis, and Seaside bentgrass, Agrostis palustris. During the past 10 to 15 years, however, fields in this area have become infested with the bentgrass nematode which caused as much as a 75% reduction in seed yield. Investigations by Courtney and Howell (1) have shown that the life cycle of the bentgrass nematode can be broken by preventing flowering of the host plant for 1 year. This principle has become the basis for several control methods devised to control the nematode in infested fields.

LIFE HISTORY OF THE BENTGRASS NEMATODE

The complete life history of the bentgrass nematode has been reviewed by Courtney and Howell (1) and by Jensen, Howell, and Courtney (3).

The primary pathological response of the plant to infection by the bentgrass nematode is the production of galls in the inflorescence. These galls replace the normal seed and contain the infective second-stage larvae. When the galls fall to the soil at maturity or are scattered by harvest operations, the contained nematode larvae are later released and re-infect any bentgrass plants growing in their vicinity. If the mature galls are kept dry, the larvae may persist in a quiescent stage for as long as 10 years.

In the field, however, the galls become softened by fall rains, permitting thousands of encased larvae to escape. Some of these larvae reach the foliar sheath of the bentgrass plants and remain adjacent to the growing points during fall and winter. When flower development is initiated in the spring, the larvae enter the embryonic ovaries and feed on the developing ovules. This activity changes the developing seeds into enlongated purplish galls. During this time the larvae pass through three molts and become adults.

One to three females and a similar number of males are generally found in each infected ovule. Each female produces as many as 1000 eggs. The adults die soon after egg laying. Within the egg larval development commences immediately and the larvae pass through the first stage and first molt within the egg. They hatch from the egg as second-stage larvae, the infective stage, after which they become dormant in the drying galls.

The nematodes will not mature and reproduce unless they can reach developing flowers of the host plant and will die if they do not reach a flower within 1 year. If the inflorescence is prevented from forming or is removed at an early stage of development, the life cycle of the nematode will be broken. Several control methods have been investigated in which the bentgrass heads have been removed by mowing and grazing or prevented from forming by summer fallowing. These methods have not proved completely effective in preventing seed formation. Growth regulating chemicals offer the possibility of flower suppression in bentgrass. An investigation of some of these materials was undertaken in 1955.

---

[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Western Washington Experiment Station. Scientific Paper No. 1969, Washington Agricultural Experiment Stations, Pullman, Washington.

[2]Respectively, Nematologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Associate Agronomist, Washington State University, and Nematologist, Crops Research Division, United States Department of Agriculture. The authors are listed alphabetically.

MATERIALS AND METHODS

Six herbicidal chemicals at various rates (Table 1) were applied to a Colonial bentgrass field severely infested with Anguina agrostis. Treatments were arranged in a randomized block design with four replications. Plots were 25 by 50 feet with 10-foot mowed alleys. All chemicals were applied in water at the rate of 100 gallons per acre.

Table 1. Herbicides applied to Colonial bentgrass, and their effect on heading, 1955.

Chemical and active ingredient per acre (pounds/application)	Dates of application	Number of heads per square foot on August 2
None (check)	--	409
2, 2-dichloropropionic acid (dalapon)		
5	May 3, June 17	19
10	May 3, June 17	0[a]
20	May 3	0[a]
30	May 3	0[a]
3-amino-1, 2, 4-triazole (amitrol)		
5	May 3, June 17	212
10	May 3, June 17	128
1, 2-dihydro-pyridazine-3, 6-dione (maleic hydrazide)		
8	May 3, June 17	24
16	May 3, June 17	7
3, 6-endoxohexahydrophthalic acid (endothal)		
8	May 3	294
16	May 3	280
3-(p-chlorophenyl)-1, 1-dimethylurea (monuron)		
8	May 3	94
16	May 3	55
4, 6-dinitro-o-sec-butyl-phenol (DNBP)		
1.25	May 3, June 17	244

[a]Bentgrass was killed by the treatment.

Data were taken during the summer of 1955 on the effectiveness of the treatments for head suppression. Four samples, each covering an area 6 x 6 inches, were taken at random from each plot and the number of heads in each sample was counted. In July 1956 each plot in the effective treatments was sampled in a similar manner and a total of 800 heads from each treatment was classified according to the percentage of florets with nematode galls. An area of 25 square feet was mowed from each of the effective treatments and the bentgrass threshed to obtain seed yield data.

RESULTS

Seed-Head Suppression in 1955: The data showing the effectiveness of the treatments in suppressing heading are presented in Table 1. Dalapon at 10, 20, and 30 pounds per acre killed the bentgrass. Endothal and DNBP had little or no effect upon bentgrass heading. Dalapon in two applications of 5 pounds per acre each, and maleic hydrazide were approximately 98% effective in preventing heading. Amitrol at both rates permitted considerable heading, but examination of galls from infected heads showed the nematodes to be dead. The monuron treatments gave spotty results, varying from complete killing to no effect within a single plot.

Head Density and Degree of Infestation in 1956: In July 1956 each plot in the effective treatments was sampled to determine the number of heads per square foot and the percentage of florets with galls (Table 2). The head density was greater on the treated plots than on the untreated. Apparently suppression of heading in 1955 stimulated heading in 1956.

The percentage infection with galls in 1956 follows closely the number of heads observed in 1955. The amitrol treatments are an exception. They permitted 25 to 50% heading in 1955, but killed the hatched larvae in the galls with the result that infestation in 1956 was very low.

Seed Yields: The seed yields (Table 3) are on the basis of approximately 90% clean seed. Treatments with a common letter in the Statistical range column do not differ significantly at the 5% level according to Duncan's multiple range test (2).

The amitrol treatments significantly outyielded the untreated check. Although the yield

Table 2. Head density of Colonial bentgrass and percentage florets with galls in 1956.

Treatment (pounds/acre)	Number of heads per square foot	% galls
Check	274	70
Dalapon, 5	532	2
Amitrol, 5	458	10
Amitrol, 10	601	4
Maleic hydrazide, 8	343	2
Maleic hydrazide, 16	508	5
Monuron, 8	512	19
Monuron, 16	413	9

Table 3. Seed yields of Colonial bentgrass in year following herbicide applications.

Treatment (pounds/acre)	Pounds of seed per acre	Statistical range
Amitrol, 10	273	a
Amitrol, 5	244	ab
Maleic hydrazide, 16	203	abc
Dalapon, 5	168	bc
Maleic hydrazide, 8	157	bc
Check	130	c
Monuron, 16	130	c
Monuron, 8	124	c

differences between the check and the dalapon and maleic hydrazide treatments were large they were not statistically significant.

DISCUSSION

The use of growth regulating chemicals to suppress heading of Colonial bentgrass and thereby break the life cycle of the bentgrass nematode is a definite possibility. The nematode was controlled to a marked degree and seed yields were increased to as much as double those of the check plots.

The immediate effect of the amitrol treatments on the bentgrass was inactivation of the chlorophyll and a resultant stunted straw-colored growth; however, many heads were still produced by the plant. Microscopic examination of infected heads from the amitrol plots showed all hatched larvae to be dead. This effect was not observed in infected heads produced in any of the other treatments. This observation indicates a possible nematocidal effect of amitrol through its systemic action. The degree of infestation in the amitrol plots in 1956 showed that some nematodes did survive, possibly as unhatched eggs.

Further experiments under more closely controlled conditions are under way to determine optimum times and rates of application of amitrol, dalapon and maleic hydrazide.

Literature Cited

1. COURTNEY, WILBUR D., and H. B. HOWELL. 1952. Investigations on the bentgrass nematode, Anguina agrostis (Steinbuck 1799) Filipjev 1936. Plant Disease Reptr. 36: 75-83.
2. DUNCAN, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.
3. JENSEN, HAROLD J., H. B. HOWELL, and W. D. COURTNEY. 1958. Grass seed nematode and production of bentgrass seed. Oregon Agr. Exp. Sta. Bull. 565. 8 pp.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE AND THE WESTERN WASHINGTON EXPERIMENT STATION

NEEDLE BLIGHT OF REDCEDAR, JUNIPERUS VIRGINIANA L.

Arthur Kelman, C. S. Hodges, and H. R. Garriss[1]

Summary

A needle blight of redcedar, Juniperus virginiana, has been observed in North Carolina, Virginia, and South Carolina characterized by an ash-brown color of affected needles, severe defoliation of lower branches, and an unusual development of juvenile needles. A fungus is associated with this disease that is considered to be Exosporium glomerulosum (Sacc.) Höhn., an organism that may be identical with certain other fungi previously reported on J. virginiana and other species of Juniperus. Optimal formation of conidia on sporodochia and best germination of conidia occurred at 20° C. The disease on redcedar in a Christmas tree plantation was controlled successfully by applications of N-trichloromethylthiophthalimide (Ortho Phaltan) at 10-day intervals and after each heavy rain.

A distinctive foliage disease affecting redcedar (Juniperus virginiana L.) has been observed over a wide geographic area in North Carolina. The first observations were made in April 1956, on young trees collected in Wayne County by Dr. Ewald Maki of the School of forestry, North Carolina State College. Subsequently, the foliage blight was observed in a plantation of trees on the Pake farm in Carteret County in 1957, where severe damage rendered the trees unsalable as Christmas trees.

During the spring and summer of 1959 additional observations were made in 12 redcedar plantations in the following North Carolina counties: Alamance, Bladen, Granville, Johnston, Onslow, Pitt, Wake and Wayne. The disease was not commonly observed in natural stands. In 1959 specimens of this disease were also collected near Ashland, Virginia and in Edgefield County in South Carolina. In February 1960 specimens of diseased redcedar foliage were collected in Columbia County, Florida, by Mr. F. R. Matthews of the U. S. Forest Service and kindly sent to the authors.

In a 7-year-old plantation comprising about 200 trees in Alamance County, approximately 10% of the trees were dead and 50% were severely defoliated. A striking characteristic of this stand was the wide variation in severity of the disease between adjacent trees. It was common for the branches of certain trees that were almost completely defoliated to be intermingled with the branches of neighboring trees that showed little or no evidence of infection. Similar variation in disease severity was observed in other plantations.

SYMPTOMS

Infected trees are characterized by a grayish-tan or ash-brown discoloration of the foliage. This is most severe on the lower leaves and progressively affects foliage higher on the tree. Defoliation of young trees may be so severe that only the tips of branches in the upper crown are still green (Fig. 1A). A distinctive characteristic of the disease is the presence of juvenile needles on larger affected branches (Fig. 1B).

Symptoms of the disease differ from those associated with the disease of redcedar caused by Phomopsis juniperovora Hahn (12). The initial symptom of Phomopsis blight is the dying-back of the tip of the stem or of a lateral branch, and as the disease progresses whole branches become involved. The needles show a light tan or reddish-tan coloration and the entire upper portion of a young tree may die. The tissue of the Phomopsis-invaded branch becomes discolored; whereas such branch discoloration is not evident in needle blight.

In general, the needle blight is most severe on young trees and it has rarely been observed causing significant damage on large trees. The most severe damage has occurred in young plantings of redcedar being grown for sale as Christmas trees.

CHARACTERISTICS OF THE FUNGUS

The sporodochium of the needle blight fungus is composed of a compact mass of olive-black

---

[1]Professor of Plant Pathology, North Carolina State College, Plant Pathologist, S. E. Forest Experiment Station, and Extension Plant Pathologist, North Carolina State College, respectively. Appreciation is expressed to Mr. F. R. Matthews for assistance in laboratory studies. Acknowledgment is also made to Mr. Edgar Pake and Mr. R. Williams, County Agent Carteret County for assistance in evaluation of fungicidal control.

FIGURE 1. A. Left--healthy redcedar from a 5-year-old plantation. Right--redcedar affected by Exosporium glomerulosum in the same plantation showing severe symptoms of needle blight.
B. Left-branchlet from healthy redcedar. Right--branchlet from redcedar affected by Exosporium glomerulosum. Note presence of juvenile needles.

mycelium which arises in the parenchyma of the needle and later becomes erumpent. The sporodochia are usually formed on the upper surface of the needle but occasionally may also be found on the lower surface. The yellow-brown conidiophores, 20-45μ x 2.5-5μ, form a compact layer over the surface of the sporodochium. The cylindrical, olive-brown, 1 to 7 septate, slightly echinulate conidia, 16.5-69.5μ x 2.0-3.6μ, are borne singly at the tips of the conidiophores. The main characteristics of this organism are essentially similar to those designated for the genus Exosporium (20). Although causal relationship of this fungus to the needle blight disease has not yet been conclusively demonstrated, present evidence indicates that the fungus described herein is the primary pathogen.

Pure cultures of the fungus were obtained from single conidia following germination of the conidia on water agar. The growth rate of the fungus was extremely slow on potato-dextrose agar and also on a Juniper needle-extract agar at 20° to 25° C. After 2 weeks the diameter of a colony on potato-dextrose agar was approximately 5 to 8 mm. Formation of conidia in culture was not obtained and for this reason it was not possible to complete inoculations with conidia from pure cultures of the fungus.

To determine optimum conditions for germination of conidia, conidial suspensions in sterile distilled water were placed in hanging drops and incubated at the following temperatures: 5°, 10°, 12°, 16°, 30°, 32°, 34°, and 36° C. Leaves containing sporodochia were also placed in Petri plates on moist filter paper at a similar range in temperatures. On the basis of readings taken after 18 hours, the most favorable temperature for conidial formation and germination was 20° with the minimum at 10° and the maximum at 34°. However, mean germ tube length 18 hours after germination of conidia was greater at 24° than at 20° or 30°.

TAXONOMIC RELATIONSHIPS OF THE CAUSAL FUNGUS

Inasmuch as the initial literature survey indicated considerable confusion as to the proper classification of the needle blight fungus, an attempt was made to abstract all pertinent references. Apparently a number of fungi with characteristics similar to the one affecting redcedar in North Carolina have been described previously. These organisms are listed in Table 1.

The first description of an Exosporium on a species of Juniperus was made by Karsten (15, 21). In 1896 Jaczewski (14) observed a foliage disease of Juniperus causing widespread damage in Smolensk and named the fungus Exosporium juniperinum.

Table 1. Fungi similar to or synonymous with the causal agent of needle blight of Juniperus virginiana.

Scientific name	Conidial measurements	Host and location	Authority
Camarosporium juniperinum Georg. & Bad.		Juniperus communis Roumania	Georgescu & Badea, 1935 (7)
Cercospora juniperina Georg. & Bad.	22-42μ x 2.2-4.8μ 1-3 septate (2-8) echinulate	Juniperus communis Roumania	Georgescu & Badea, 1937 (8)
Cercospora sequoiae Ell. & Ev.	40-70μ x 4-6μ 3-5 septa	Sequoia gigantea Pennsylvania	Ellis & Everhart, 1887 (6)
Cercospora sequoiae Ell. & Ev. var. juniperi Ell. & Ev.	15-30μ x 3μ	Juniperus virginiana and J. communis var. depressa Wisconsin	Ellis & Everhart, 1887 (6)
Clasterosporium glomerulosum Sacc.	35-45μ x 8-10μ 6-7 septate	Juniperus communis Germany	Saccardo, 1881 (18)
		Juniperus communis Munich, Germany	Allescher, 1895 (1)
Coryneum juniperinum Ell.	35-40μ x 6-8μ 6 septate	Juniperus communis Iowa	Ellis, 1882 (5)
Exosporium betheli Dearn.	35-51μ x 6-8.5μ 8 celled	Juniperus communis California	Dearness, 1929 (4)
Exosporium deflectens Karst.	14-20μ x 5-6μ 3 septate	Juniperus communis Finland	Karsten, 1888 (15)
Exosporium deflectens Karst. (emend. Sandu-ville)	23-42μ x 5-6μ 1-4 septa	Juniperus communis Roumania	Sandu-ville, 1939 (22)
Exosporium glomerulosum (Sacc.) Höhn.	35-43μ x 7-9μ 6-7 septa	Juniperus communis Istria (Italy)	v. Höhnel, 1909 (13)
Exosporium juniperinum Jacz.	20-40μ x 5-7μ 3-6 septa	Juniperus communis Smolensk, Russia	Jaczewski, 1901 (14)
Pleospora conglutinata Goebel		Juniperus communis Schweinfurt, Germany	Goebel, 1879 (9)
Sporidesmium glomerulosum Sacc.	35-45μ x 8-10μ 6-7 septa	Juniperus communis Germany	Saccardo, 1878 (17)

Subsequently, von Höhnel (13) described a fungus collected on J. communis and named it Exosporium glomerulosum (Sacc.) Höhn. He recognized that it was similar to a fungus on Juniperus communis initially described as Sporidesmium glomerulosum Sacc. by Saccardo. In subsequent reports Saccardo (18, 19) reclassified this fungus and placed it in the genus Clasterosporium.

Von Höhnel also concluded that the fungus described by Goebel (9) as the conidial stage of Pleospora conglutinata was identical with E. glomerulosum.

Although he overlooked the paper by Jaczewski, von Höhnel considered Coryneum juniperinum to be synonymous with E. glomerulosum.

Furthermore, von Höhnel suggested that E. deflectens (15) probably was synonymous with

E. glomerulosum although there were discrepancies in spore size.

The first report of a fungus on J. virginiana with characteristics similar to the fungus currently prevalent on this species in North Carolina was Cercospora sequoiae Ell. & Ev. var. juniperi Ell. & Ev. (2). This fungus was described in 1887 by Ellis and Everhart (6) on specimens sent to them from Wisconsin by J. J. Davis.

Subsequently, Davis (3) collected specimens of Cercospora sequoiae Ell. & Ev. on J. communis L. var. alpina Gaud. at Wind Lake, Wisconsin.

A similar foliage disease of J. communis was reported in Roumania by Georgescu and Badea (7) and they described the organism as a new species, Camarosporium juniperinum Georg. & Bad. In a subsequent paper (8) they reclassified the fungus as another new species, Cercospora juniperina Georg. & Bad.

In 1937 Sandu-ville (22) collected specimens of J. communis showing a foliage blight and concluded that the fungus was a species of Exosporium as defined originally by Link in 1809 (20) and designated the fungus as E. deflectens.

In describing a destructive foliage blight of oriental arborvitae (Thuja occidentalis L.), Plakidas (16) concluded that the fungus previously described on juniper did not appear to be a Cercospora since the fruiting body could definitely be classified as a sporodochium. He suggested that the fungus originally reported on juniper from Wisconsin probably was a species of Exosporium.

Specimens of the arborvitae fungus collected by Dr. Plakidas were examined by Dr. Chupp (2) at Cornell. He found that the conidia were echinulate and similar to those of C. sequoiae and C. sequoiae var. juniperi which he had examined previously. It was Chupp's opinion that these fungi were essentially very similar and he raised a question as to whether they properly belonged in the genus Cercospora because of the echinulation of the conidia.

More recently a Cercospora-like fungus was reported on the foliage of Juniperus virginiana near Kenosha, Wisconsin by Greene (10, 11), who noted the suggestion by Plakidas that this fungus was not properly assigned to the genus Cercospora and suggested that the fungus should be designated on a provisional basis as Exosporium deflectens. Greene also obtained similar material from Michigan that was labelled as E. glomerulosum (Sacc.) Höhn.

Examinations were made of specimens kindly sent by Dr. R. F. Cain from the herbarium of the University of Toronto labelled Exosporium glomerulosum (Sacc.) Höhn. on Juniperus communis var. depressa collected in Ontario, Canada by R. F. Cain in 1954 and 1957, on Juniperus communis collected in Michigan by E. B. Mains and B. B. Kanouse in 1933, and on this same host in Latvia by J. Smarods in 1937. Although conidia were sparse, the sporodochia and conidiophores present appeared to be identical with reproductive structures of Exosporium present on Juniperus virginiana in North Carolina.

Collections of material in Wisconsin on J. communis made by Dr. H. C. Greene and on J. virginiana in Arkansas in 1959 by Dr. C. Wilson were compared with material obtained in North Carolina on J. virginiana. The measurements are presented in Table 2. The measurements of conidia from Arkansas and North Carolina are quite similar to each other but different from the material collected in Wisconsin. However, the nature of the fruiting body and shape of conidia and conidiophores were essentially similar for all three collections.

Table 2. Conidial measurements of Exosporium from three different geographic areas.

Host	Location	Conidial measurements (in μ) length	Conidial measurements (in μ) width	Number of septa mean	Number of septa range
Juniperus virginiana	North Carolina	40.8 $\pm$ 11.1	3.1 $\pm$ 0.4	3	1-7
Juniperus virginiana	Arkansas	41.4 $\pm$ 7.4	3.4 $\pm$ 0.2	3	1-5
Juniperus communis	Wisconsin	19.9 $\pm$ 14	3.3 $\pm$ 0.2	2	1-4

In order to resolve questions as to the proper identity of the species of Exosporium on redcedar and also to determine whether this fungus can attack all the hosts that have been reported, it will be necessary to complete inoculation experiments on all the species considered as hosts and compare spore size in relation to host plant affected. Until these and related questions can be resolved, it is suggested that the fungus on redcedar be designated as E. glomerulosum. It is also suggested that the other species listed in Table 1 may be considered as synonyms.

CONTROL

During the spring and summer of 1958 attempts to control needle blight were made in a Christmas tree planting on the farm of Edgar Pake in Carteret County. Only one material, Ortho Phaltan 50 W (N-trichloromethylthiophthalimide) manufactured by California Spray-Chemical Corporation was used in this preliminary experiment. The dosage was 2 pounds per 100 gallons of water. Spray applications were made with a 3-gallon compressed air sprayer at 10- to 14-day intervals and repeated after heavy rains during the period of May 23 to September 16. Nine applications of approximately 6 gallons of spray per application were made to 58 trees ranging from 3 to 8 feet in height.

Excellent control of the disease on current season foliage was obtained. All sprayed trees remained green and were in salable condition for Christmas trees. Unsprayed trees were severely damaged and not in salable condition. Additional research with other fungicides and with various schedules is needed since it is unlikely that redcedar Christmas tree plantings in North Carolina will warrant the extensive spray schedule used in this preliminary test.

Literature Cited

1. ALLESCHER, A. 1895. Mykologische Mittheilungen Aus Süd-Bayern. 1113. Clasterosporium glomerulosum Sacc. Hedwigia 34: 256-292.
2. CHUPP, C. 1954. A monograph of the fungus genus Cercospora. Ithaca, New York. 667 pp.
3. DAVIS, J. J. 1909. Fourth supplementary list of parasitic fungi of Wisconsin. Trans. Wisconsin Acad. Sci. 16: 739-773.
4. DEARNESS, J. 1929. New and noteworthy fungi. VI Mycologia 21: 326-332.
5. ELLIS, J. B. 1882. New species of North American fungi. Bull. Torrey Botan. Club 9: 133-134.
6. ELLIS, J. B., and B. M. EVERHART. 1887. Additions to Cercospora, Gloeosporium and Cylindrosporium. J. Mycology 3: 13-22.
7. GEORGESCU, C. U., and M. BADEA. 1935. Caderea acelor de Juniperus cauzatu de o ciuperca noua Camarosporium juniperinum Georg. et Badea nov. sp. Rev. Padurilor (Bucuresti) 47: 155-162.
8. GEORGESCU, C. U., and M. BADEA. 1937. O nova specie de Cercospora pe ace de Juniperus. Cercospora juniperina. Georg. et Bad. nov. sp. Anal. Inst. Cercet. Expt. For. (Bucuresti) 1: 37-47.
9. GOEBEL, K. 1879. Pleospora conglutinata als Ursache der Erkrankung und Nadelschütte von Juniperus communis. Jahreshefte Vereins Vaterl. Naturk. Württemberg 35: 305-312.
10. GREENE, H. C. 1946. Notes on Wisconsin parasitic fungi IX. Trans. Wisconsin Acad. Sci. 38: 235-248.
11. GREENE, H. C. 1952. Notes on Wisconsin parasitic fungi. XV Amer. Midlands Nat. 48: 37-54.
12. HAHN, G. G., C. G. HARTLEY, and R. G. PIERCE. 1917. A nursery blight of cedars. J. Agr. Research 10: 533-539.
13. HÖHNEL, F. von. 1909. Fragmente zu Mykologie. VI. Sitzungsber. Kais. Akad. Wissensch. 118: 275-443.
14. JACZEWSKI, A. L. 1901. Über eine Pilzkrankheit auf dem Wachholder. Zeitschr. Pflanzenkr. 11: 205-209.
15. KARSTEN, P. A. 1888. Symbolae ad Mycologiam Fennicam. Fragmenta mycologica XXIII. Hedwigia 27: 258-260.
16. PLAKIDAS, A. G. 1945. Blight of oriental arborvitae. Phytopathology 35: 181-190.
17. SACCARDO, P. A. 1878. Sporidesmium glomerulosum. Michelia 1: 131.
18. SACCARDO, P. A. 1881. Fungi Italici. Hyphomyceteae. Clasterosporium glomerulosum. Plate 746.
19. SACCARDO, P. A. 1886. Clasterosporium glomerulosum. Sylloge Fungorum 4: 392.
20. SACCARDO, P. A. 1886. Exosporium Link. Sylloge Fungorum 4: 755.
21. SACCARDO, P. A. 1892. Exosporium deflectens Karst. Sylloge Fungorum 10: 653.
22. SANDU-VILLE, C. 1939. Exosporium deflectens Karst. Auf Blättern von Juniperus communis L. in Rumänien. Bull. Sect. Sci. Acad. roum. 21:113-116.

DEPARTMENT OF PLANT PATHOLOGY, NORTH CAROLINA STATE COLLEGE, RALEIGH, NORTH CAROLINA

A SIMPLE AND INEXPENSIVE TRACTOR-MOUNTED INOCULUM APPLICATOR[1]

Wayne R. Sitterly and Guy L. Buckner, Jr.[2]

Summary

A simple and inexpensive tractor-mounted inoculum applicator is described. This applicator, which is attached to the drawbar of a tractor and which consists of a 10-gallon stainless steel tank, a small power-take-off driven air compressor and a hose, wand and nozzle from a knapsack sprayer, has been used successfully to inoculate plants with both viruses and fungi. The speed and uniformity of application have been increased greatly.

INTRODUCTION

Because of the large number of individual plants involved, any plant breeding procedure that is adaptable to mass production principles will materially reduce the time and labor involved in a specific project. This paper describes inoculation equipment designed for the mass inoculation of large numbers of plants under field conditions.

MATERIALS AND CONSTRUCTION

The major components of the inoculator are a 10-gallon stainless steel tank mounted vertically on an L-shaped frame, and a small air compressor driven from the power-take-off of the tractor. These components are mounted on a rectangular steel base plate bolted to the drawbar of the tractor. Views of the assembled unit are shown in Figures 1 and 2. The total cost of materials used was approximately $70.00

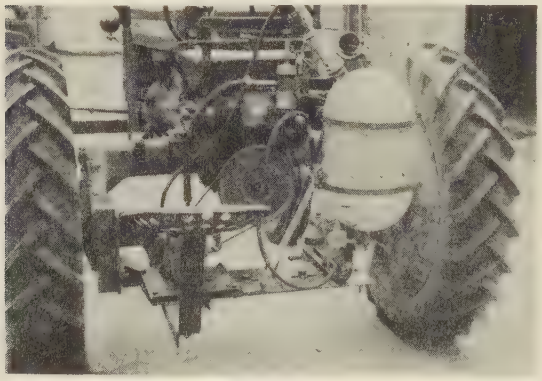


FIGURE 1. Rear view of tractor-mounted inoculum applicator. The tank is on the right and the compressor on the left, partly obscured by the seat.

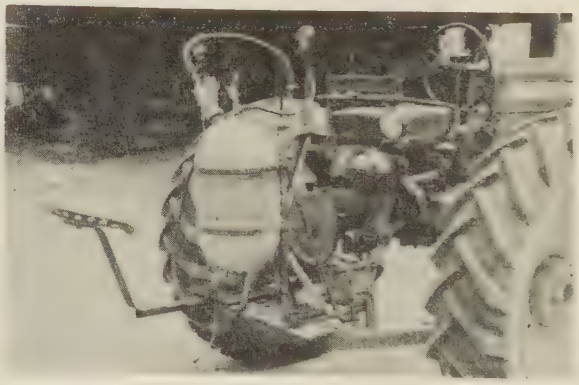


FIGURE 2. Side view of tractor-mounted inoculum applicator.

The tank (a hydrogen container obtained from military surplus) contains an outlet on the bottom and an air pressure safety valve and a Y-shaped inlet on top. The Y-shaped inlet was connected to the tank by drilling an off-center hole into the top of the tank. A 1 1/2-inch stainless steel close nipple inlet pipe was placed in the hole and was welded to the tank. After the entire weld was coated with stainless solder to insure against air leaks, a Y-shaped plumber's joint was attached. One arm of the Y-shaped inlet is used for filling, while the other is attached to the air compressor.

The vertical frame for holding the tank is a 3-inch steel channel approximately 3 feet long. Two 1/4-inch thick steel bands were welded to this channel so that they could be placed around

[1]Technical contribution No. 336, South Carolina Agricultural Experiment Station, Clemson College, Clemson, South Carolina.

[2]Assistant Plant Pathologist and Horticulture Assistant, Clemson College Truck Experiment Station, Charleston, South Carolina.

the tank and drawn tightly by bolts through holes drilled in their ends. The channel was then placed in a vertical position and welded to the steel base plate.

Air pressure is provided by a small compressor bolted to the base plate and driven by the tractor power-take-off through a V-belt drive with a 2 to 1 speed increase. The compressor is a Bendix-Westinghouse automotive air brake compressor with a capacity of 7.1 cubic feet per minute, also obtained from military surplus. A spring-loaded rubber seal and the compressor pulley were placed on the crankshaft. The compressor base was tapped, and a pipe inserted to provide a means of lubrication.

Compressed air is fed to the inoculum tank through a 1/2-inch pressurized rubber hose. A trap containing steel wool was placed in the line between the compressor and the tank to catch any oil which may have vaporized in the compressor. On the tank end of this hose a piece of plastic tubing was attached by means of flange fittings and inserted to the bottom of the tank, to provide agitation to the inoculum suspension. The air pressure in the tank is controlled by a regulator set to cut off the compressor at the desired pressure. Two pressure gauges are utilized, one to indicate tank pressure and the other to indicate the pressure at the outlet. The present apparatus utilized the hose, wand, and nozzle from a knapsack sprayer, although it may be modified for one-man operation by extending the rubber outlet hose to a one-row fixed boom mounted under the tractor.

A metal hay-rake seat was welded to the base plate for the convenience of the operator.

DISCUSSION

Most methods of field scale plant inoculation are laborious and lack uniformity because of the necessity of providing a constant pressure. This is especially true with virus inoculations, since a moderate pressure is required to score the leaves with carborundum and permit adequate penetration of the virus particles into the leaf cells. A sufficiently high and uniform pressure cannot be maintained with a standard knapsack sprayer for more than 200 linear feet of row. A manually drawn inoculator, pressured by bottled carbon dioxide, is heavy and slow.

With the inoculator described herein set at 60 p.s.i., a 2-acre squash field was inoculated with squash mosaic virus in 40 minutes. This operation required 3 to 4 hours with equipment and facilities previously used. Inoculation of the same acreage of cucumbers with anthracnose, at 10 p.s.i., formerly required 1 hour. These examples illustrate the range, versatility, and potential of this inoculator. Visual observation of disease symptoms produced, actual lesion counts, and microscopic examination of carborundum-scored leaves attest to the uniformity of application. Expenditure of time has been cut as much as 80% below previous field scale inoculation methods. This equipment should be particularly beneficial to plant breeders and phytopathologists.

CLEMSON COLLEGE TRUCK EXPERIMENT STATION, CHARLESTON, SOUTH CAROLINA

PHYSALOSPORA OBTUSA (CKE.) WINTER ON SOUTH CAROLINA LIVE OAKS

Wayne R. Sitterly[1]

Trunks and scaffold limbs of several live oaks (Quercus virginiana) in the vicinity of Charleston, South Carolina were observed to be infected with Physalospora obtusa (Cke.) Winter in the early spring of 1959. Current season lesions were characterized by a rusty reddish-brown color. Although typical brown conidia were most frequently observed, numerous hyaline to pale yellow ascospores were noted in early February. The ascospores were spherical and uninucleate when first formed in the ascus, later becoming cylindrical and multinucleate. This appears to be the first report of P. obtusa on live oaks in South Carolina, and perhaps also on the eastern seaboard.

CLEMSON COLLEGE TRUCK EXPERIMENT STATION, CHARLESTON, SOUTH CAROLINA

[1]Assistant Plant Pathologist.

RELATIONSHIP BETWEEN INJURY BY THE CLOVER ROOT CURCULIO AND INCIDENCE OF FUSARIUM ROOT ROT IN LADINO WHITE CLOVER

J. H. Graham and R. C. Newton[1]

Abstract

Results of two experiments conducted in the greenhouse suggest a relationship between injury, mechanical and that caused by larvae of the clover root curculio (Sitona hispidula (F.)), and the increased incidence of Fusarium root rot in Ladino white clover.

Stands of Ladino white clover in northeastern United States usually are seriously depleted during the second or the third year of growth. Reasons for the lack of persistence of this perennial are not known, but an insect-injury-disease complex is believed to be involved. A previous study[2] indicated a relationship between root injury (mechanical and that caused by root-feeding insects) and incidence of crown and root rot of red clover.

Experiments were conducted in the greenhouse during the winters of 1958-59 and 1959-60 to determine the effects of the clover root curculio (Sitona hispidula (F.)), Fusarium spp., and mechanical injury, separately and in combination, on growth and on incidence of root rot in Ladino white clover.

MATERIALS AND METHODS

In the 1958-59 experiment, 24 one-gallon crocks were filled with a greenhouse-potting soil and steamed for 8 hours at 5 pounds' pressure. Two-month-old seedlings of Ladino white clover were transplanted one to a crock and grown in a greenhouse at 20° to 25° C and at 13-hour daylength. After 2 weeks the plants were given the following treatments: 1) control, 2) plant rootlets injured mechanically with a scalpel, 3) holes made in soil near plants and filled with inoculum consisting of three isolates of Fusarium spp. (isolated from Trifolium spp.) and grown on sand-corn meal agar, 4) plants mechanically injured and Fusarium added to soil, 5) 20 field-collected adult clover root curculios caged on plants to infest the soil with their eggs, 6) 20 field-collected adult curculios caged on plants with Fusarium added to the soil. Each treatment was replicated four times. All plants were caged 7 weeks to produce uniform shading and were then allowed to wilt between waterings.

The test was repeated in the winter of 1959-60 as before except that Fusarium was mixed with the soil before transplanting the seedlings, replications were increased to five, and two seedlings were transplanted in each crock. In addition, to favor disease development the plants were placed in a growth chamber at 27° C for the final 10 days of the experiment. The plants in both experiments were dug at the end of 11 weeks and the following data noted: 1) number of stolons, 2) root vigor, 3) incidence of root rot, and 4) plant fresh-weight.

RESULTS

In both experiments all treatments reduced stolon number and root vigor in comparison with the control (Table 1). Plant weights declined proportionately to the treatments: 1) addition of Fusarium to soil, 2) Fusarium plus mechanical injury, 3) clover root curculio, and 4) Fusarium plus curculio, respectively. Reduced growth in the curculio treatments was due in part to defoliation by the adults. Stripping of small rootlets and gouging of the tap roots were evident on plants in crocks containing larval progeny of the adult curculio.

Total plant weights were consistently less and root rot was more severe in the 1959-60 test than in the 1958-59 test.

In the 1958-59 experiment most root rot developed in the treatments Fusarium plus mechanical injury and Fusarium plus curculio. An analysis of the data in the 1959-60 test indicated that plants injured mechanically both with and without Fusarium and those exposed to curculio plus Fusarium developed significantly more rot than the control plants. In both experiments little rot developed in plants in the curculio treatment and almost none in the controls.

[1]Plant Pathologist, Crops Research Division, and Entomologist, Entomology Research Division, respectively, Agricultural Research Service, United States Department of Agriculture.

[2]Graham, J. H., and R. C. Newton. 1959. Relationship between root feeding insects and incidence of crown and root rot in red clover. Plant Disease Reptr. 43: 1114-1116.

Table 1. Stolon number, root vigor, plant weight, and root-rot incidence of Ladino white clover under different treatments in the greenhouse.

Treatment	Number of stolons		Root vigor[a]		Plant weight (g)		Root-rot incidence[b]	
	1958-59[c]	1959-60[d]	1958-59	1959-60	1958-59	1959-60	1958-59	1959-60
1. Control	8	4	9	7	73	25	0	16
2. Mechanical injury	6	3	8	5	76	23	0	44
3. *Fusarium* in soil	6	3	8	4	65	21	0	24
4. *Fusarium* + mechanical injury	7	3	7	4	55	20	13	61
5. Curculios	6	3	7	3	49	11	5	24
6. *Fusarium* + curculios	5	2	5	3	38	10	28	45

[a]Based on index 1-10 (10 most vigorous).
[b]Estimated percentage of root area showing rot.
[c]Average of four replications, one plant per crock.
[d]Average of five replications, two plants per crock.

UNITED STATES REGIONAL PASTURE RESEARCH LABORATORY, UNIVERSITY PARK, PENNSYLVANIA

THE OCCURRENCE OF CHLOROGENIC ACID IN STRAWBERRY PLANTS AFFECTED WITH CRINKLE AND YELLOWS VIRUSES[1]

Richard D. Durbin, B. S. Castillo, and T. H. King[2]

The current procedure for detecting strawberry viruses involves grafting the plant to be tested to an indicator plant, usually Fragaria vesca (7). The utilization of a simple, rapid chemical test as an alternative to grafting has merit, provided that it is a dependable indication of virus presence in the majority, if not all, of the commercial varieties and, if possible, enables one to detect the majority of viruses found in strawberry. Although chemical tests have been utilized in selected instances (1,3, 6), the technique has not been widely applied. A study was undertaken to ascertain whether chemical differences exist in strawberry which might serve as a basis for this type of test.

Initially, extracts from F. vesca plants that were virus free or infected with either crinkle or yellows viruses were analyzed by paper chromatography. The most significant difference found among the three extracts was the presence under UV light of a blue-white fluorescent spot on the chromatograms of sap from diseased plants. In the presence of ammonia vapor the color changed to green. In daylight this spot became yellow when sprayed with 1% $NaNO_2$ in 10% aqueous acetic acid, then reddish when subsequently sprayed with 1N NaOH.

The unknown compound was partially purified by two-dimensional chromatography using amyl alcohol: acetic acid: water (4:1:5) and 10% aqueous isopropyl alcohol as the two solvent systems. It was tentatively identified as an isomer of chlorogenic acid on the bases of the color reactions and its Rf in five solvent systems, 10% aqueous isopropyl alcohol, n-butyl alcohol: acetic acid: water (4:1:5), 10% aqueous acetic acid, amyl alcohol:acetic acid:water (4:1:5), and water. Alkaline hydrolysis of the compound yielded products which were chromatographically similar to caffeic and quinic acids and to the hydrolytic products of chlorogenic acid itself.

Testing for the presence of chlorogenic acid throughout this work was done in the following manner: 3 gm fresh weight of leaves and/or crowns per 2 ml of distilled water were ground rapidly using a mortar and pestle and the sap filtered through 2 layers of cheesecloth. Sand was added as an abrasive when necessary. The liquid was briefly heated to boiling, then centrifuged. Ten lambda of the supernatant fraction was applied on Whatman No. 1 filter paper strips, and 10% aqueous acetic acid used as the developing solvent. After drying, the strip was sprayed with the previously mentioned Hoepfner's reagent, 1% $NaNO_2$ in 10% aqueous acetic acid followed by 1N NaOH. Due to interfering substances in the plant extract, the Rf of the chlorogenic acid is somewhat smaller than that of pure chlorogenic acid. Although other natural tannins also react to give colored nitroso derivatives, they can be differentiated from chlorogenic acid by their chromogenic and Rf properties (8, 9). None of these interfering compounds has as yet been found to affect the assay in strawberries.

Extension of the study to nine commercial varieties that were known to be infected with crinkle and yellows viruses showed that they also contained chlorogenic acid. Only two commercial varieties, Robinson and Premier, together with several numbered seedling selections were available for further testing of crinkle- and yellows-free plants. Under the conditions employed, no chlorogenic acid could be detected in any of these plants. Extracts from F. vesca plants infected with either latent A or Frazier's mild mottle viruses did not, however, give a positive test for chlorogenic acid.

Whether the presence of chlorogenic acid is a valid criterion of virus infection depends upon two conditions: first, that chlorogenic acid is not present in virus-free strawberry plants and, second, that it is always produced when the plant is infected. Information on the former point is difficult to ascertain, since many commercial varieties of strawberry are virus-infected. On the basis of the evidence presented here, it appears that the presence of chlorogenic acid in the vegetative portions of strawberry is associated with the presence of either crinkle or yellows viruses, but not with latent A or Frazier's mild mottle viruses.

An increase in the chlorogenic acid content, as well as other polyphenols, in response to fungus (4, 5) or virus (2) infection has been noted in other plants. It is suggested that this increase in polyphenol content may contribute to our inability to mechanically transmit certain viruses easily.

---

1 Paper No. 4354, Scientific Journal Series, Minnesota Agricultural Experiment Station.

2 Assistant Professor, Rockefeller Foundation Fellow, and Professor, respectively, Department of Plant Pathology and Botany, University of Minnesota.

Literature Cited

1. FIFE, J. M., and I. E. STOKES. 1959. Amino acid changes identify ratoon stunting disease. Sugar y Azucar 54 (12): 27-28.
2. GEISSMAN, T. A. 1956. The flavonoid constituents of normal and virus-infected peach and cherry leaves. Arch. Biochem. Biophys. 60: 21-26.
3. KENKNIGHT, GLENN. 1951. The acid test for phony disease of peach. Phytopathology 41: 829-832.
4. KIRKHAM, D. S. 1959. Host factors in the physiology of disease. 110-118. In Plant Pathology Problems and Progress. 1908-1959. University of Wisconsin Press.
5. KUĆ, J., R. E. HENZE, A. J. ULLSTRUP, and F. W. QUACKENBUSH. 1956. Chlorogenic and caffeic acids as fungistatic agents produced by potatoes in response to inoculation with Helminthosporium carbonum. J. Am. Chem. Soc. 78: 3123-3125.
6. McANELLY, C. W., M. G. PAYNE, and J. L. FULTS. 1956. Detection of potato leaf roll by paper chromatography and electrophoresis. Am. Potato J. 33: 134-140.
7. MILLER, P. M. 1958. Comparative efficiency of excised leaf-petiole grafts and stolon grafts for transmitting certain strawberry viruses. Plant Disease Reptr. 42: 1043-1047.
8. REEVE, R. M. 1951. Histochemical tests for polyphenols in plant tissues. Stain Tech. 26: 91-96.
9. SWAIN, T. 1953. The identification of coumarins and related compounds by filter-paper chromatography. Biochem. J. 53: 200-208.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY,
INSTITUTE OF AGRICULTURE, UNIVERSITY OF MINNESOTA,
ST. PAUL, MINNESOTA

DIFFERENTIATION OF OAT HELMINTHOSPORIA BY THE RAGDOLL METHOD[1]

S. S. Ivanoff

Abstract

A clear-cut differentiation in symptom expression was made between Helminthosporium avenae and H. victoriae by the ragdoll method, described earlier by this writer for testing resistance to Victoria blight. The high reliability of this method is determined by the fact that the symptoms thus induced by the two species are practically identical in all details with those induced by them on young plants in the field. A collection of 29 Helminthosporium cultures isolated from oats were tested on eight varieties by this method. H. victoriae caused complete disintegration of the radicles of very young seedlings of susceptible varieties like Alamo, Arlington, and Victorgrain 48-93 after 5 days in the ragdoll at 28° C and 100% relative humidity, but did not affect the coleoptiles. On the other hand, under the same conditions H. avenae induced characteristic necrotic lesions and streaks on the coleoptile, rotting of the base of the culm, and black spots on the first true leaf, after 10 to 12 days in the ragdoll. The radicle became discolored but did not disintegrate until much later, if at all. It was established that thus far no Helminthosporium isolate, new or old, has been found capable of inducing Victoria blight on such resistant varieties as Mid-South and Southland, or on Delair, Nortex 107, or Delta Red 88; but all of these oats, together with blight-susceptible Alamo, Arlington, and Victorgrain 48-93, were subject to leaf blotch and showed the well-know symptoms induced by H. avenae. These results were confirmed by greenhouse inoculation tests. Two "culm rot" cultures, included in the collection, pathogenetically behaved much like H. avenae. The relationship between these two pathogens is not clear and needs further investigation.

A great concern has been shown in recent years by plant pathologists, county agents, and growers in general in the southern United States about diseases of oats caused by various Helminthosporium fungi. Three of these diseases are of primary importance: leaf blotch (H. avenae), Victoria blight (H. victoriae), and "culm rot" (pathogen not named)[2]. There has been some question as to the best way to recognize and differentiate these troubles and their respective pathogens. Difficulties have been encountered in the differentiation of the various symptoms on the differnt parts of the host during its life cycle, and by the variability in morphology of the isolates concerned.

Recently Earhart (2) attempted to differentiate pathogenic forms of Eu-Helmithosporium species which attack oats[3]. He devised a laboratory procedure employing 10-day-old seedlings placed in test tubes, adequately illuminated and containing mycelial agar suspensions. After 24 hours, two main symptom expressions, "blighting" and "rotting", were recorded on four varieties of oats, Arlington, Victorgrain 48-93, Mid-South, and Southland. Assuming that these symptom expressions were those of Victoria blight he concluded that a strong majority of isolates in his fungus collection belonged to the species H. victoriae and induced "Victoria blight" even on oats like Mid-South and Southland which are known to be resistant to this disease. A much smaller group of isolates included those forms which were capable of "blighting" Southland but no other variety. These isolates he believed to be "culm rot" incitants. He stated that some of the cultures had the ability to incite both diseases, that is, Victoria blight and "culm rot" (host varieties not specified). The same investigator concluded that a clearer understanding of the relationship among the various groups of fungi studied should be left for subsequent studies.

---

[1] Journal paper (New Series) No. 873, Mississippi Agricultural Experiment Station.

[2] According to Earhart (1), who first reported and described this condition on Southland oats, the causal agent of "culm rot" had certain characteristics common to H. sativum and others to H. victoriae but "pathologically it does not fit either species." According to the same author the "culm rot" fungus induced foliage symptoms similar to those caused by H. victoriae.

[3] Nishikado (6) established Eu-Helminthosporium as a group having long, elliptical, obclavate fusiform conidia, more or less tapered towards both ends, and germinating only from the polar cells (with Ophiobolus ascigerous stage). Another group, Cylindro-Helminthosporium, has cylindrical conidia which germinate from both polar and intercalary cells (Pyrenophora or Pleospora sexual stage).

REASONS FOR UNDERTAKING THESE STUDIES

Most workers realize that a clearer understanding of these problems is very much needed, not only from a purely pathological point of view, but also for the benefit of the plant breeder. Another specific and important question is whether new strains of H. victoriae have arisen which could cause Victoria blight on such a highly resistant variety as Mid-South which was developed by the writer (4) and which had been previously tested against a large number of H. victoriae isolates obtained from all over the country, including several from South Carolina. Proof of the existence of such strains is necessary, because if Victoria blight could be induced on Southland and other well-known resistant oats which do not have the Victoria gene for resistance to crown rust, then our concept of the relationship between these two diseases will have to be re-examined and explained anew.

The particular problem for investigation was to test as many Helminthosporium isolates as possible, including those of Earhart's, on a number of well-known varieties of diverse genetic composition, and to clearly differentiate by new and old techniques between those inducing Victoria blight and isolates inducing other diseases which might be confused with Victoria blight.

Table 1. Kind of disease symptoms induced by 29 Helminthosporium cultures isolated from oats and tested on 8 varieties by the laboratory ragdoll and greenhouse inoculation methods. (Varieties tested: Alamo, Arlington, Delair, Delta Red 88, Mid-South, Nortex 107, Southland, and Victorgrain 48-93)

Designation	Origin of culture	Disease symptoms induced in laboratory	Disease symptoms induced in greenhouse
1. H. victoriae	Standard culture M 224 (check)	Victoria blight[a]	Victoria blight
2. H. victoriae	Miss. 76, from oat foliage (check)	do.	do.
3. Earhart 11	South Carolina	do.	do.
4. Earhart 1	do.	none	--
5. H. avenae	Luttrell 989, Georgia (check)	leaf blotch	leaf blotch
6. H. avenae	Luttrell 1392, Georgia (check)	do.	--
7. Earhart 15	South Carolina	do.	leaf blotch
8. Earhart 22	do.	do.	--
9. Earhart 27	do.	do.	--
10. Southland 1	seed, var. Southland, Florida	do.	--
11. Southland 2	do.	do.	leaf blotch
12. Southland 3	do.	do.	--
13. Southland 4	do.	do.	--
14. Southland 5	do.	do.	--
15. Southland 6	do.	do.	--
16. Southland 7	do.	do.	--
17. Southland 8	do.	do.	--
18. Southland 9	do.	do.	--
19. Southland 10	do.	do.	--
20. S.C. 16-2	seed with leaf blotch, C.I. 7136, Miss.	do.	--
21. S.C. 16-3	do.	do.	--
22. S.C. 16-4	do.	do.	--
23. S.C. 16-10	do.	do.	--
24. S.C. 16-11	do.	do.	leaf blotch
25. S.C. 16-13	do.	do.	--
26. S.C. 16-22	do.	do.	--
27. S.C. 16-23	do.	do.	--
28. G 3-1	"culm rot" culture	do.	--
29. H 25	do.	do.	--

[a]Victoria blight symptoms induced only on Alamo, Arlington, and Victorgrain 48-93; the other five varieties remaining unaffected. Leaf blotch symptoms were induced on all 8 varieties with cultures 5 through 29.

MATERIALS AND METHODS

The cultures tested were isolated from diseased oat material as indicated in Table 1. Most of the cultures were subsequently purified by single-spore isolation. The group also included the five (out of six) of Dr. Earhart's type cultures used in his investigation. These were, according to his designation, Nos. 1, 11, 15, 22, and 27. Culture No. 16 ("culm rot") was not available. Checks were two cultures each of H. victoriae and H. avenae of proven high pathogenicity, producing conidia of more or less typical morphology and inducing, respectively, Victoria blight and leaf blotch on oats.

The 8 varieties on which all of the cultures were tested in the laboratory, and also in the greenhouse, were Alamo, Arlington, Victorgrain 48-93 -- all known to be susceptible to Victoria blight -- and Delair, Delta Red 88, Mid-South, Nortex 107, and Southland, all known to be resistant to Victoria blight.

The ragdoll method was adapted to differentiate H. victoriae from H. avenae and the "culm rot" pathogen. The results of the laboratory tests were verified by greenhouse inoculations by spraying young plants with mycelial suspensions, gently rubbing the leaves afterwards, and keeping the plants under bell jars at about 24° to 30° C.

TEST FOR VICTORIA BLIGHT

With the ragdoll method the seeds were dipped in an agar culture (2 weeks or older), grown on PDA, diluted with water, 1:2, and blended in a Waring Blendor for 20 to 30 seconds. The thoroughly wet ragdolls were incubated at 27° to 28° C at 100% relative humidity. They were examined after 5 days. Cultures capable of producing Victoria blight caused disintegration of seedling roots after 5 days on susceptible varieties such as Alamo, Arlington, and Victorgrain 48-93, and no damage was caused to the coleoptiles (Fig. 1). On the other hand, no symptoms were noted on roots or coleoptiles on resistant varieties, such as Delair, Delta Red 88, Mid-South, Nortex 107, and Southland or any other blight resistant oat (Fig. 1A). (If H. avenae were to be present in the ragdoll, no effect would be shown on the young seedlings at this time.)

TEST FOR LEAF BLOTCH

Leaf blotch symptoms on roots, coleoptiles, and the first true leaf were noted on seedlings in the ragdoll 10 to 12 days after incubation, the time interval depending on the degree of virulence of the H. avenae culture employed. The first symptom was on the coleoptile, appearing as one or more streaked dark brown to black spots[4]. Later, dark-discoloration of the culm just above the kernel may appear, eventually causing rotting of the young culm (Fig. 1B, C, and D). The first true leaf may show spots very similar to those appearing in the coleoptile (Fig. 1B). These are like the lesions shown on the first leaf of diseased seedling oats in the field. The roots begin to decay usually starting near the kernel, and the decay extends toward the tips, until eventually the whole root system turns chocolate brown. A common sign in the ragdoll test for leaf blotch is the formation of "arborescent tufts" by the fungus on the decayed parts of the seedling. They appear as small white pustule-like formations, described by Turner and Milland (8) in cultures of H. avenae on certain artificial media.

Most laboratory tests were made with eight replications and usually repeated three or more times. The greenhouse tests were in four replications and repeated twice. All tests included standard "check" cultures of H. victoriae and H. avenae of known history and proven pathogenicity.

RESULTS AND CONCLUSIONS

With the ragdoll method, a clear differentiation was made between the action of H. victoriae on one hand and H. avenae type cultures on the other. Symptoms of the two seedling diseases were like those found on young seedlings in the field. This differentiation was also confirmed by greenhouse tests.

Victoria blight symptoms were induced only by the two standard H. victoriae cultures used as checks and by Earhart culture No. 11, and only on Alamo, Arlington, and Victorgrain 48-93.

[4] O'Brien and Dennis (7) in describing the seedling symptoms of leaf blotch in the field stated: "It is probably true to say that the coleoptile stage of infection necessarily precedes all the others and is the only phase which is invariably present."

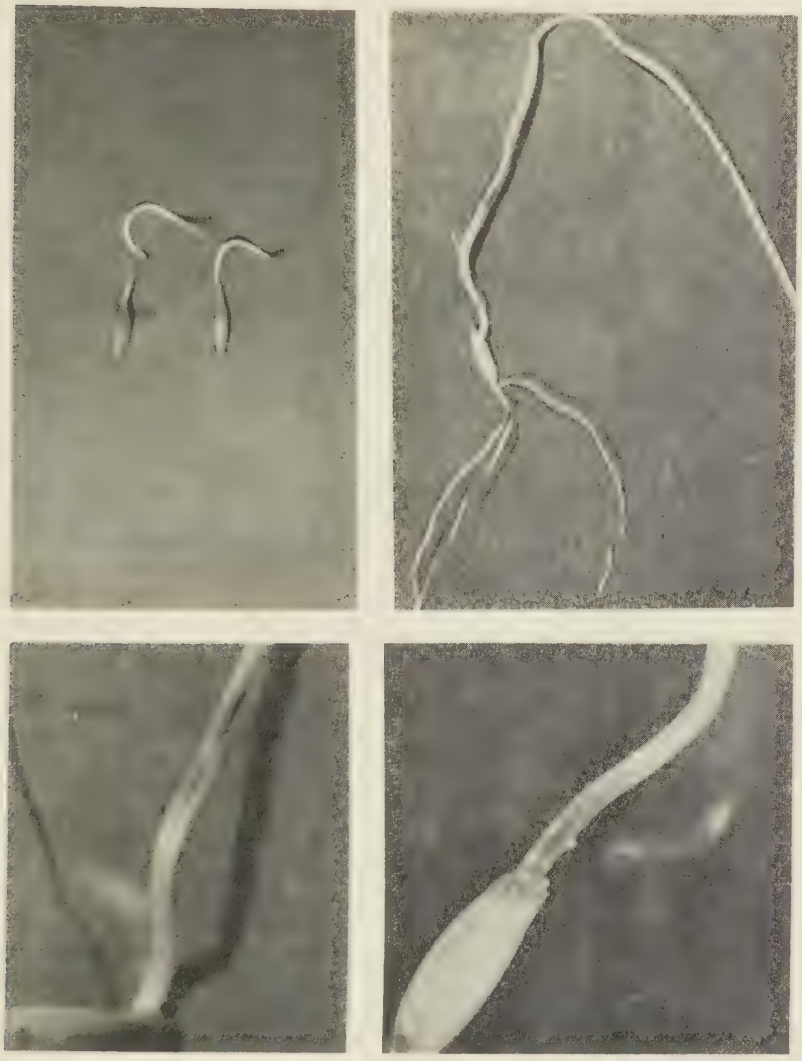


FIGURE 1. Differentiation between Victoria blight (H. victoriae) and leaf blotch (H. avenae) of oat seedlings by the ragdoll method: A -- Victoria blight on susceptible oats, 5 days after placing seeds in ragdoll. Note disintegration of roots and absence of lesions on coleoptiles. B -- Initial leaf blotch symptoms 8 to 12 days in the ragdoll. Note a lesion on coleoptile and two necrotic spots on primary leaf. C -- Details of leaf blotch lesion on coleoptile, enlarged. D -- Rotting of culm in leaf blotch, beginning above the kernel. Note fungus tufts on rotting tissues.

Victoria blight symptoms were induced only on Alamo, Arlington, and Victorgrain 48-93, and only by isolates 1, 2, and 3 (Table 1). The varieties Delair, Delta Red 88, Mid-South, Nortex 107, and Southland remained unaffected. Leaf blotch symptoms were induced on all eight varieties with cultures No. 5 through No. 29.

No such symptoms were induced on Delair, Delta Red 88, Nortex 107, Mid-South, and Southland by these or any of the 29 cultures tested. These results are at variance with those reported by Earhart who stated that certain of his "H. victoriae" isolates that he worked with, and which were included in these studies, were pathogenic to Mid-South as well as to Victorgrain 48-93. No Victoria blight symptoms were induced on Mid-South or Southland with any of Earhart's cultures by the ragdoll method or by the greenhouse method. Mid-South remained unaffected by any of the numerous additional Helminthosporium cultures collected from various parts of the United States against which it has been tested during and after the time it was selected as a Victoria-blight resistant mutant from the variety Victorgrain 48-93 (4).

All eight varieties exhibited symptoms of leaf blotch (H. avenae) when subjected to the action of certain of the isolates (Nos. 5 through 29, which included Earhart Nos. 15, 22, 27, Table 1). There were marked degrees of pathogenicity among these isolates.

No differences were noted in symptoms induced in the laboratory by the "check" H. avenae cultures on one hand and the two "culm rot" cultures, on the other. It would be desirable to make such a differentiation, if possible. (The "culm rot" cultures were old, not too highly pathogenic, and were not tested in the greenhouse in the comparative trials.) The relationship between leaf blotch and "culm rot" diseases requires a more thorough investigation.

The lack of agreement between the results and interpretations of Earhart and this writer may be explained: 1) by the different methods of testing -- Earhart's method, which has its merits, has not been verified by other well-tried methods of inducing Victoria blight or differentiating between that blight and leaf blotch; 2) by the non-inclusion of other Victoria blight resistant oats, such as Delair or Nortex 107, to serve as additional checks against Earhart's method; and 3) by the fact that some of the cultures classified by Earhart as H. victoriae actually seemed to be closer to H. avenae and to some other forms -- judging by the morphology of the conidia -- and induced leaf blotch instead of Victoria blight symptoms on all the eight varieties employed.

Because of the great variability in spore morphology within and among some isolates this writer concurs with the opinion of some investigators that the ability to induce a certain well-defined disease should be one of the chief bases for classification of these Helminthosporium fungi.

Literature Cited

1. EARHART, R. W. 1951. Helminthosporium crown and culm rot of oats. (Abst.) Phytopathology 41: 11.
2. EARHART, R. W. 1959. The differentiation of pathogenic forms of Eu-Helminthosporium species which attack oats. Plant Disease Reptr. 43: 1184-1187.
3. IVANOFF, S. S. 1951. Mass screening in oat breeding. J. Hered. 42: 224-230. [not cited in text]
4. IVANOFF, S. S. 1957. The Mid-South oat variety, a Victoria-blight resistant mutant. J. Hered. 48: 101-107.
5. IVANOFF, S. S. 1959. Studies on Helminthosporium leaf blotch of oats (Pyrenophora avenae). (Abst.) Phytopathology 49: 318. [not cited in text]
6. NISHIKADO, Y. 1928. Studies on the Helminthosporium disease of Gramineae in Japan. Ohara Inst. Agr. Research. Spec. Rept. 4: 1-394.
7. O'BRIEN, D. G., and R. W. G. DENNIS. 1930. The Helminthosporium disease of oats. West of Scotland Agr. College Res. Bull. 3: 1-73.
8. TURNER, D. M., and W. A. MILLAND. 1931. Leaf spot of oats, Helminthosporium avenae. (Bri. and Can.)Eidam. Ann. Appl. Biol. 18: 535-558.

DEPARTMENT OF PLANT PATHOLOGY AND PHYSIOLOGY, MISSISSIPPI STATE UNIVERSITY, STATE COLLEGE, MISSISSIPPI

PHYTOPHTHORA ROOT ROT OF SOYBEANS AS AFFECTED BY SOIL TEMPERATURE AND MELOIDOGYNE HAPLA[1]

Thomas D. Wyllie[2] and Donald P. Taylor[3]

Summary

Inoculation of Harosoy soybeans with Phytophthora sojae[4] and Meloidogyne hapla caused more severe symptoms, as expressed by plant height, dry weight, and post-emergence loss in stand, than either pathogen alone. The fungus was the primary cause of the damage with the nematode acting as a contributing factor. Damage caused by these organisms was most severe at soil temperatures above 20° C and increased as the temperature increased.

INTRODUCTION

In recent years plant parasitic nematodes have been demonstrated to affect several fungus diseases of plants (1, 2, 3, 4, 5, 7, 9). The relationship of nematodes to fungus diseases of soybean, however, is not generally known. Taylor and Wyllie (8) demonstrated that the incidence of pre-emergence damping-off of soybeans, caused by Rhizoctonia solani Kuehn, was greatly increased by the presence of Meloidogyne hapla Chitwood, 1949 and somewhat increased by the presence of M. javanica (Treub, 1885) Chitwood, 1949. The experiment described was made to study the effect of M. hapla on Phytophthora root rot of soybeans at several soil temperatures.

MATERIALS AND METHODS

Phytophthora sojae Kaufmann & Gerdemann[5] was grown on a vermiculite-lima bean-broth mixture at room temperature (22° to 25° C) for 10 days. The inoculum was thoroughly mixed with steamed soil and placed in 6-inch pots in controlled soil-temperature tanks. Steamed soil was used in the treatments not containing the fungus. Fifteen seeds of the soybean variety Harosoy were planted in each pot.

Second-stage infective larvae of M. hapla were obtained from infected greenhouse-grown Marglobe tomato plants. Aqueous suspensions of 2500 larvae per pot were poured over the seeds at the time of planting.

Tank temperatures were maintained by a continuous influx of cold water (approximately 16° C) combined with an auxiliary heating element regulated by a thermostatically controlled mercury switch. Eight such tanks were utilized in this experiment: two each at 15° to 20°, 20° to 25°, 25° to 30°, and 30° to 35° C. Treatments of P. sojae, M. hapla, M. hapla plus P. sojae, and non-inoculated check were replicated in each tank. The experiment was terminated after 6 weeks.

Root-knot ratings were made by counting the number of galls produced on several 1-inch-long root sections and are expressed as the average number of galls per gram of root (dry-weight basis).

RESULTS AND DISCUSSION

Results are given in Figures 1, 2, 3, and 4. The effect of temperature on the severity of P. sojae as measured by plant height, dry weight, and post-emergence mortality increased as the temperature increased, except at the highest temperature range (30° to 35° C). At this temperature range plant height and dry weight of the P. sojae inoculated treatments were well below

---

[1]Paper No. 4336, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2]Research Fellow, Department of Plant Pathology and Botany, University of Minnesota; also Agent, Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

[3]Instructor, Department of Plant Pathology and Botany, University of Minnesota.

[4]Synonymous with Phytophthora megasperma Drechs. var. sojae nov. var. as named by A. A. Hildebrand. Can. J. Botany 37: 927-957.

[5]The authors express their appreciation to Dr. John Tuite, Purdue University, Lafayette, Indiana for supplying cultures of P. sojae.

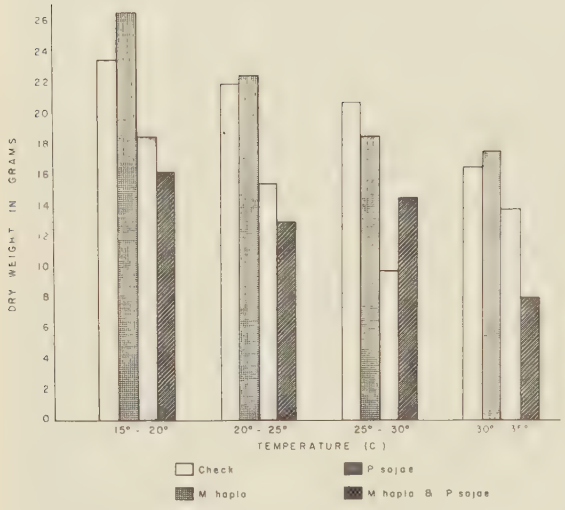


FIGURE 1. Effects of Phytophthora sojae and Meloidogyne hapla, alone and in combination, on whole-plant dry weight at four temperature ranges. (Average of four replicates.)

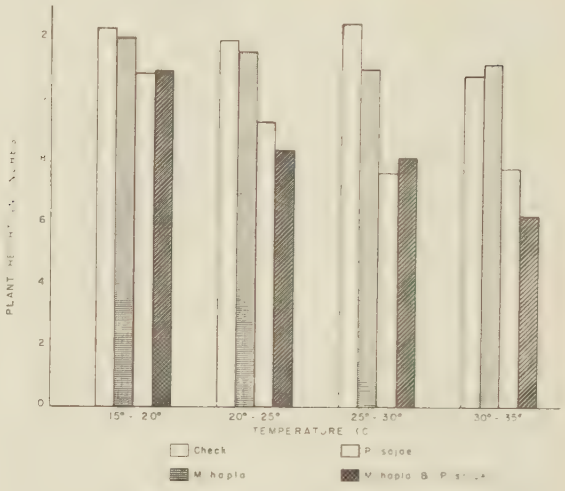


FIGURE 2. Effects of Phytophthora sojae and Meloidogyne hapla, alone and in combination, on plant height at four temperature ranges. (Average of four replicates.)

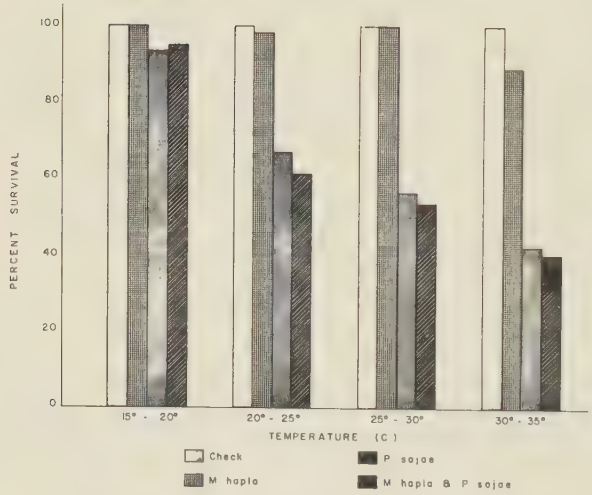


FIGURE 3. Effects of Phytophthora sojae and Meloidogyne hapla, alone and in combination at four temperatures, on post-emergence mortality expressed as percentage survival of emerged plants. (Average of four replicates.)

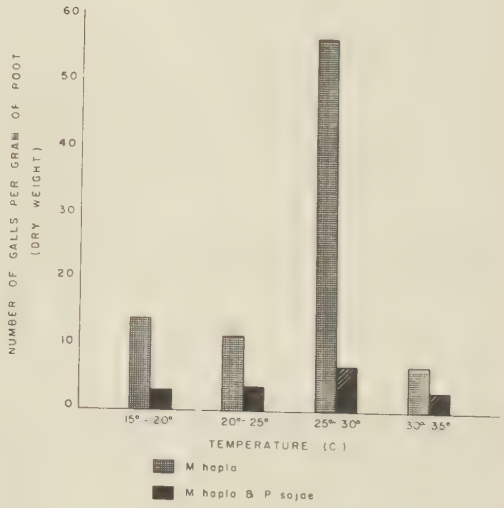


FIGURE 4. Effect of four temperature ranges and inoculation with Phytophthora sojae on root-knot gall formation. (Average of four replicates.)

the non-inoculated checks, but the differences between the two treatments were not so great as those observed at 25° to 30°. Although the highest post-emergence mortality occurred at 30° to 35°, surviving plants were slightly taller and had greater dry weight than those maintained at 25° to 30°. The smallest differences in plant height and dry weight between P. sojae inoculated and non-inoculated treatments were obtained at the lowest temperature range (15° to 20°). The greatest effects of P. sojae were obtained above 25°, and the optimum temperature range for pathogenicity of this organism was 25° to 30° or 30° to 35°, depending on the criteria selected.

In treatments containing both M. hapla and P. sojae, soybeans were more severely affected than in the treatments inoculated with the fungus alone, with two exceptions: at the lowest temperature range (15° to 20° C), plant height and post-emergence mortality were almost the same in the two treatments; and at 25° to 30° plant dry weight was considerably higher and plant height slightly greater in the treatment containing both organisms. The reason for these two reversals from the general effect of the addition of M. hapla is unknown, but at 25° to 30°, P. sojae alone has the maximum effect on these measurements and the number of root-knot galls per unit of weight was also the highest. The increase in plant weight in the P. sojae plus M. hapla treatment might be related to the fact that M. hapla stimulates root proliferation in soybean (6).

The increase in severity in the treatments consisting of both organisms was seldom more than additive, and on this basis M. hapla is considered to be only contributory to the case of P. sojae infections.

Soil temperatures ranging from 25° to 30° C were apparently optimum for M. hapla as measured by gall production in the treatments with nematodes alone (Fig. 4). The number of galls produced at this temperature range in the fungus-nematode treatment was only slightly higher than that produced at other temperature ranges. Since equal quantities of nematode inoculum were used in both treatments, it is assumed that similar numbers of infections occurred. However, since many of the roots in the fungus-nematode treatment were severely rotted, the actual number of galls observed was low.

Literature Cited

1. HOLDEMAN, Q. L. 1956. The effect of the tobacco stunt nematode on the incidence of Fusarium wilt in flue-cured tobacco. Phytopathology 46: 129.
2. HOLDEMAN, Q. L., and T. W. GRAHAM. 1954. Effect of the sting nematode on expression of Fusarium wilt in cotton. Phytopathology 44: 683-685.
3. JENKINS, W. R., and B. W. COURSEN. 1957. The effect of root-knot nematodes, Meloidogyne incognita acrita and M. hapla, on Fusarium wilt of tomato. Plant Disease Reptr. 41: 182-186.
4. LOOS, C. A. 1959. Symptom expression of Fusarium wilt disease of the Gros Michel banana in the presence of Radopholus similis (Cobb, 1893) Thorne, 1949 and Meloidogyne incognita acrita Chitwood, 1949. Proc. Helminthol. Soc. Wash. D. C. 26: 103-111.
5. REYNOLDS, HAROLD W., and R. G. HANSON. 1957. Rhizoctonia disease of cotton in presence or absence of the cotton root-knot nematode in Arizona. Phytopathology 47: 256-261.
6. SASSER, J. N. 1954. Identification and host-parasite relationships of certain root-knot nematodes (Meloidogyne spp.). Maryland Agr. Exp. Sta. Bull. A-7(Tech.). 31. pp.
7. SASSER, J. N., G. B. LUCAS, and H. R. POWERS, Jr. 1955. The relationship of root-knot nematodes to black-shank resistance in tobacco. Phytopathology 45: 459-461.
8. TAYLOR, D. P., and T. D. WYLLIE. 1959. Interrelationship of root-knot nematodes and Rhizoctonia solani on soybean emergence. (Abst.) Phytopathology 49: 552.
9. THOMASON, IVAN J. 1958. The effect of the root-knot nematode, Meloidogyne javanica, on blackeye bean wilt. (Abst.) Phytopathology 48: 398.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY, INSTITUTE OF AGRICULTURE, UNIVERSITY OF MINNESOTA, ST. PAUL, AND CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

A GENETIC ABNORMALITY IN AN IDAHO CLONE OF FRAGARIA VESCA[1]

A. W. Helton[2]

Summary

Native strawberry plants which displayed prominent symptoms similar to, but less chlorotic than, those of leaf variegation in commercial varieties were collected and tested for symptom stability and virus content using standard greenhouse procedures. From these tests it was concluded that the abnormality was not infectious, under present definitions of the term, but was closely related to the described genetic leaf variegation conditions in commercial varieties, though more stable and more clonally uniform in effect than generally encountered elsewhere.

INTRODUCTION

Decline of strawberry fields has been a recognized problem in Idaho since 1951 (4, 5).

During the 10 years prior to 1951, considerable progress was made by a number of workers in developing methodology for detection of latent strawberry viruses (3, 7, 8). Clones of Fragaria vesca, both European and American, received most attention (3, 8); but others such as F. virginiana (7) also were used as sensitive indicator plants. Both of these were known to be common in Idaho.

Preliminary investigations utilizing F. vesca indicator plants revealed that the decline problem in Idaho was traceable to latent virus infection as well as to previously recognized root rotting fungi. Plants were collected from a variety of environments and indexed in a greenhouse. Rarely were virus symptoms recognized in field plants. However, the iron-complex deficiency symptom frequently was found, and occasionally a leaf variegation symptom was encountered. As the survey continued, the role of native plants in disseminating viruses in some localities came to be a question that could not be ignored. Consequently the survey was broadened to include such plants.

With one exception, sampling of native strawberry plants in the foothills and mountains surrounding cultivated areas revealed only fungous disorders. This exception was an unusual symptom having visual characteristics both of virus infection and genetic variegation. Experiments were designed to determine the nature of this condition.

MATERIALS AND METHODS

The abnormal suspect, or donor, plants first were collected from a roadside canyon north of Cascade, Idaho in March 1954. Two types of plants were growing in the immediate vicinity, only one of which exhibited the abnormal condition, and then only in a relatively small area. Plants were collected from this site and brought into a greenhouse during March of each year throughout the 6 years of the investigation. Each time the collected plants were planted in a standard greenhouse soil mixture consisting of two parts soil, one part sand, and one part manure. The mixture was shredded thoroughly in a shredding machine and pasteurized in an electric pasteurizer prior to potting of the plants. Supplementary nitrogen was supplied at 3-month intervals by adding small quantities of nitrapils (33.3% nitrogen) to the pots.

Latent virus indicator plants were grown from seeds and runners and potted in the same soil mixture. The ages of both donor and indicator plants were purposely different for each test, so as to detect any transmissible agent whatever the requisite age conditions of the plants. Most of the indicator plants were of the East Malling clone of F. vesca, although in the earlier years of the tests dual grafts were made simultaneously to plants of F. vesca and a F. vesca x alpine hybrid as a check of relative efficiency of the two indicators under the conditions of the tests. The latter soon were discarded in favor of the vesca clone. The reasons for this were many; runners were not produced by the hybrid to facilitate grafting, in duplicated tests with culture complexes of latent viruses vesca plants reacted more often and more prominently, and the hybrid was not adapted to propagation by vegetative means.

Supplementary light was supplied for 2 hours near midnight during the 1954, 1955 and 1956 tests. Mixed banks of incandescent and fluorescent lamps were suspended in various combinations 3 feet above the greenhouse benches. This procedure was discarded after 1956 because of the difficulty experienced in keeping the suspect clone in an actively growing condition during

---

[1] Approved by the Director of the Idaho Agricultural Experiment Station as Research Paper No. 494.

[2] Associate Plant Pathologist, Idaho Agricultural Experiment Station.

the winter months even with the added light. Instead, grafts were made in spring and early summer.

Grafting was accomplished with razor blades and elastic rubber electricians' tape, employing the commonly used tongue graft. Runners were used if present. If not, either petioles or flower scapes were grafted with the method used for runners. During 1959 only the excised leaf technique (1) was used. Painting of grafts with waxes was tried in earlier tests but abandoned because results were not improved substantially. Grafted plants were not kept in moist chambers. The test for organic union was the ability of the graft to support a severed donor plant for 7 days.

RESULTS

Each spring, when a new supply of the abnormal plants was collected, the symptoms and general condition of plants in the vicinity were noted.

Leaflets were minutely crinkled and streaked with pale chloroses roughly in the direction of the veins (Fig. 1). Leaflets were more or less wedge shaped at their bases and arched slightly downward.

FIGURE 1. Typical leaves of the abnormal native clone of F. vesca var. bracteata with a normal leaf of the East Malling clone of F. vesca (center).

FIGURE 2. A plant of the abnormal clone of F. vesca var. bracteata (right) beside a healthy plant of F. vesca, East Malling clone.

FIGURE 3. Two runner plants of the abnormal clone of F. vesca var. bracteata after being graft-sustained on East Malling F. vesca plants for 3 weeks.

This downward arching became prominent after growth for some weeks in the greenhouse. Affected leaves often were smaller than leaves of unaffected plants, but often also they were fully as large (Fig. 2). Runners were fewer but larger in diameter and had pronounced red pigmentation. General plant vigor was less in the natural habitat but approximately equal to unaffected plants in the greenhouse during the spring and summer months. Viability of seed was considerably less than that of other native strawberry clones and species. Considerable difficulty was experienced in obtaining successful grafts of donor runners to indicator plants (Fig. 3).

In general, the symptoms of the suspect plants more nearly approximated those described as due to genetic variegation, or June yellows. This condition has received attention from numerous workers, as indicated by Darrow's (2) review of the literature in 1955. However, certain differences between the leaf variegation condition reviewed by Darrow and the Idaho abnormality appear to exist under both field and greenhouse conditions. For example, symptoms in the Idaho clone are reliably predictable. All progeny plants developed prominent symptoms at all times under all conditions to which they were exposed. Occasionally affected plants developed leaflets in the greenhouse that were nearly normal in appearance, but this was infrequent. Another difference was that affected plants did not continue to deteriorate as indicated by an increase in chlorosis with time. A uniform relationship was maintained between degree of chlorosis, which was minor, and normal green. The crinkling, or ruffling, of the leaves also was reasonably uniform under all conditions. Thus the sport, or mutation, was substantially more constant than reported for the leaf variegation condition in commercial varieties.

Identification of affected plants proved difficult because of their uniformly abnormal condition. However, with the help of Dr. William H. Baker[3] and Dr. J. L. McMullen[4], it was concluded that affected plants constituted a mutated clone of F. vesca var. bracteata.

As illustrated in Table 1, all index tests were failures insofar as virus transmission was

Table 1. Grafting experiments which failed to induce symptoms in latent virus indicator plants.

Date	Number of donor-indicator plant pairs[a]	Indicator plants used	Type of graft	Criteria proving organic union[b]
6/15/54	3	Vesca; V x A	runner to runner runner to scape	---
8/17/54	3	Vesca; V x A	runner to petiole; petiole to scape	2 runner plants graft sustained for 9 days
4/7/55	6	Vesca; V x A	runner to petiole	---
5/5/55	6	Vesca; V x A	runner to runner; runner to petiole	1 runner plant graft sustained for 7 days
4/21/56	8	Vesca	runner to petiole	---
5/28/57	4	Vesca	runner to runner	---
5/24/58	10	Vesca	runner to runner or runner to petiole	2 runner plants graft sustained for 18 days
6/24/58	9	Vesca	runner to runner	2 runner plants graft sustained for 27 days
3/30/59	12	Vesca	excised leaflets	6 leaflets graft sustained for 10 days
5/22/59	10	Vesca	excised leaflets	10 leaflets graft sustained for 35 days

[a] Minimum of three grafts per pair.
[b] After 3 weeks donor plants were severed from indicator plants where other than excised leaf grafts were used, sufficient organic union for transmission of viruses being assumed if detached portions lived on the indicator plants for 7 days.

concerned. Despite the fact that viruslike particles have on occasion been associated with conditions of leaf variegation in strawberry plants (9), the exhaustive tests conducted as a direct part of this investigation and as parts of related investigations failed to transmit any visually detectable symptoms to indicator plants.

[3] Head, Department of Biological Sciences, University of Idaho.
[4] Department of Botany, University of Idaho.

DISCUSSION AND CONCLUSIONS

The apparent mutation found in a clone of Fragaria vesca var. bracteata, as herein described, demonstrated unusual symptom stability and uniformity throughout every affected plant. Symptomatically the clone was very similar to the commonly described leaf variegation conditions of various descriptions and names throughout the 5-year test period. The symptoms also resembled certain viroid conditions in strawberries, particularly when they were manifested in plants grown under greenhouse conditions. A degree of chlorotic mottling developed under those conditions reminiscent of the symptoms induced by many viruses and virus complexes. However, no part of the symptom complex was transmitted to any indicator plants to which affected plants were grafted. Thus the condition cannot be attributed to infective agents as they are presently defined.

The conclusion is justified, therefore, that the abnormal condition in the suspect plants is a genetic abnormality of a type closely related to previously described but less stable leaf variegations.

Literature Cited

1. BRINGHURST, R. S., and VICTOR VOTH. 1956. Strawberry virus transmission by grafting excised leaves. Plant Disease Reptr. 40: 596-600.
2. DARROW, G. M. 1955. Leaf variegation in strawberry -- a review. Plant Disease Reptr. 39: 363-370.
3. HARRIS, R. V., and MARRY E. KING. 1942. Studies in strawberry virus diseases. V. The use of Fragaria vesca L. as an indicator of yellow edge and crinkle. J. Pomol. and Hort. Sci. 19: 227-242.
4. HELTON, A. W. 1954. Small fruit diseases and their control. Idaho Agr. Exp. Sta. Bull. 219. 42 pp.
5. HELTON, A. W. 1952. The strawberry root rot problem. Idaho Agr. Exp. Sta. Cir. 124. 2 pp.
6. HELTON, A. W. 1956. Strawberry plant production aided by station project. Idaho Agr. Sci. 41(2): 2. (not cited in text)
7. HILDEBRAND, A. A. 1941. The reaction of Fragaria virginiana to the virus yellow-edge. Can. J. Research 19: 225-233.
8. MILLER, P. W. 1951. Sensitivity of some species of Fragaria to strawberry yellows and crinkle virus diseases. Plant Disease Reptr. 35: 256-261.
9. THORNBERRY, H. H., A. E. VATTER, and D. M. BEESON. 1951. Virus-like particles in strawberry plants with foliar variegation. (Abst.) Phytopathology 41: 35.

UNIVERSITY OF IDAHO AGRICULTURAL EXPERIMENT STATION, MOSCOW

A STRAIN OF TOBACCO MOSAIC VIRUS ASSOCIATED WITH A WILT AND STEM NECROSIS OF MATURE, FIELD-GROWN BELL PEPPER[1]

Harry H. Murakishi and Shigemi Honma[2]

Abstract

A strain of tobacco mosaic virus was isolated from mature pepper plants affected with wilt and stem necrosis. Wilt symptoms were reproduced in the greenhouse on Penn Wonder-Allbig and Sunnybrook plants 7 to 8 weeks following inoculation.

During August of 1956, bell pepper plants (Capsicum annum) bearing mature fruits were found wilting on the Horticulture Farm at East Lansing. The only plants affected were from segregating F_3 lines of a Penn Wonder-Allbig cross which was hybridized for certain desirable plant and fruit characters. From a total of 90 plants of these lines in a replicated, randomized plot, 72 succumbed to the disorder during a period of 2 weeks. Other varieties in the same experimental plot included Morgold, California Wonder, Vinedale and Pimiento, which were not affected. All plants were growing in adequate soil moisture, but the Penn Wonder-Allbig plants wilted suddenly as if from drought or from the effects of a wilt organism. The dark green foliage showed no other symptom than flagging of the leaves and petioles. When stems were sectioned longitudinally, extensive brown, necrotic areas were found in the pith. The stem lesions as well as portions of the roots and petioles were cultured but no bacteria or fungi were isolated. A plant which had recently wilted was dug, potted in soil and brought to the greenhouse. It was pruned severely and placed under partial shade. Within 2 weeks new leaves appeared which were lighter in color and showed a distinct mottle.

A wilt of Tabasco pepper caused by tobacco etch virus (TEV) was described by Greenleaf (1). From his descriptions, the sudden onset of wilting in Tabasco pepper caused by TEV appeared similar to those observed by the writers in the Penn Wonder-Allbig F_3 lines. A mottled leaf from the affected plant was triturated in water and the inoculum was applied on leaves of several host plants at the 4 to 6 leaf stage. The results are summarized in Table 1. Seven

Table 1. Results of inoculation with a strain of TMV isolated from pepper.

Host and variety	Main symptoms[a]
PEPPER	
Penn Wonder-Allbig	SN, TN, W
Penn Wonder	SN, TN
Allbig	LNS only
California Wonder	SN
Hungarian Sweet	SN
Long Red Cayenne	SN, TN
Pimiento	SN
Red Chilli	SN, TN
Serrano	SN, TN
Sunnybrook	SN, TN, W
Tabasco	LL only
Nicotiana glutinosa	LL only
Cucumber, National Pickling	N.S.
Zinnia, Pom Pom	N.S.
Bean, Pinto	N.S.
Scotia	N.S.

[a]SN = stem necrosis; TN = top necrosis; LNS = local necrotic symptoms; LL = local lesions; W = wilt and N.S. = no symptoms.

[1]Journal Paper Number 2634. Michigan Agricultural Experiment Station, East Lansing. The writers thank Dr. F. O. Holmes of the Rockefeller Institute for Medical Research; Dr. W. H. Greenleaf, Department of Horticulture, Alabama Polytechnic Institute; and Dr. R. P. Kahn of the United States Plant Introduction Station, Glenn Dale, Maryland for seeds of host plants.

[2]Respectively, Associate Professor of Plant Pathology and Associate Professor of Horticulture.

FIGURE 1. Left -- wilt symptoms induced in Sunnybrook pepper seedling, 8 weeks after inoculation with TMV. Right -- healthy, uninoculated control.

days after inoculation stem necrosis was produced on seedlings of the Penn Wonder-Allbig F_3 lines, Penn Wonder, Sunnybrook, Red Chilli, Serrano, Long Red Cayenne, Hungarian Sweet, California Wonder and Pimiento. Seven to 8 weeks after inoculation surviving Penn Wonder-Allbig and Sunnybrook plants developed wilt symptoms (Fig. 1) similar to those observed in the field. Although a few necrotic local lesions appeared on leaves of Allbig, no stem lesions or wilt symptoms developed. Local lesions only were observed on Tabasco, Datura stramonium and Nicotiana glutinosa. Cucumber, zinnia, and Pinto and Scotia bean remained symptomless. Since no wilt symptoms developed on Tabasco, the results indicated that the virus involved was not TEV but a strain of tobacco mosaic virus (TMV). The lack of symptoms on bean suggested, however, that this was not the common strain of TMV which incites local lesions on these two varieties. For further confirmation on the identity of the virus involved, infective juice heated to 90° C for 10 minutes and then cooled was rubbed on leaves of two N. glutinosa plants. After 4 days a few lesions appeared. These were cut out, triturated in 1% K_2HPO_4 buffer and rubbed on leaves of Samsun tobacco which developed a systemic mottle within 7 to 10 days. Sub-inoculations from infected Samsun to Penn Wonder-Allbig plants reproduced the stem necrosis and wilt symptoms.

Holmes (2) indicated that certain pepper varieties are extremely susceptible to TMV and respond with a fatal necrosis in the greenhouse as shown in his studies on inheritance of resistance to TMV in pepper. Similar symptoms have been observed by the writers on certain susceptible varieties of pepper in the greenhouse, but the occurrence of a wilt of mature, field-grown plants due to TMV is believed rather uncommon. The susceptibility to stem necrosis and wilt in Penn Wonder-Allbig plants was probably derived from the female parent. Allbig, the male parent, showed no stem lesions or wilt when inoculated with the strain of TMV, while Penn Wonder was severely affected.

Literature Cited

1. GREENLEAF, W. H. 1953. Effects of tobacco-etch virus on peppers (Capsicum sp.). Phytopathology 43: 564-570.
2. HOLMES, F. O. 1937. Inheritance of resistance to tobacco-mosaic disease in the pepper. Phytopathology 27: 637-642.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, DEPARTMENT OF HORTICULTURE, MICHIGAN STATE UNIVERSITY, EAST LANSING

EFFECT OF SOIL FUNGICIDES UPON SOIL-BORNE PLANT PATHOGENIC BACTERIA AND SOIL NITROGEN

Donald E. Munnecke[1] and John Ferguson[2]

The question frequently is raised as to whether the chemicals commonly used as soil fungicides are also effective against soil-borne plant pathogenic bacteria. In some reports bacteria have survived extremely high doses of a toxicant, but in others they have succumbed to relatively low doses. For example, Mai et al.(4), using dried inoculum on toothpicks fumigated in a large pressure cooker, were unable to kill Corynebacterium sepidonicum with a dose of 6 pounds of methyl bromide per 100 cubic feet of space. MacLachlan et al. (3) failed to kill the same organism with fumigations of ammonia, chlorine, chloropicrin, hydrogen sulfide, methyl bromide or carbon disulfide (50, 100, 150 mg/l for 8 or 16 hours), although ethylene oxide was toxic. Richardson (7) exposed dried cultures of C. sepidonicum on pieces of jute to 20% (by volume) of methyl bromide or 2% ethylene oxide before obtaining a lethal dose. When McKeen (2) buried in soil green tomato fruits infected with Pseudomonas tomato and pepper leaves infected with Xanthomonas vesicatoria and treated them with methyl bromide, he found that X. vesicatoria was destroyed by doses less than 2 pounds/100 cubic feet, whereas P. tomato survived a dose of 4 pounds/100 cubic feet.

Experiments on the effect of fungicides on the soil nitrogen cycle have frequently demonstrated (1) that nitrification is inhibited, but ammonification is usually not greatly affected by fungicide treatment. Recovery of nitrification is slow and may take up to 8 weeks after treatment.

Since the reports of the bactericidal effects of soil fungicides were conflicting and the reported experiments were often made in the absence of soil, the studies reported here were initiated to determine the approximate doses required to control soil-borne bacterial plant pathogens in soil under conditions approximating those found in California nurseries. Methods of treatment of soil were similar to those used commercially. In addition, the effect of treatments on soil nitrogen was investigated.

MATERIALS AND METHODS

The testing procedure involved burying pieces of infected host tissue in plastic screen bags beneath 2 inches of soil, treating the soil with fungicides for appropriate periods, retrieving the test pieces and testing for bacterial viability by inoculating susceptible host plants with infusions of the treated material. Results were evaluated on the basis of the presence or absence of disease in inoculated host plants. Four experiments were conducted during a period of 1 year.

Infected host tissue for fungicide treatment was prepared by inoculating tomato stems with pure cultures of Agrobacterium tumefaciens or Corynebacterium michiganense and by inoculating stems of Pelargonium hortorum with Xanthomonas pelargonii. When wilting and vascular discoloration were produced by infection by the two vascular parasites, or when galls 1 1/2 inches in diameter were produced by A. tumefaciens, samples were taken as follows: for C. michiganense, 2-inch pieces of stem; for X. pelargonii, 2-inch pieces of stem, split once lengthwise; and for A. tumefaciens, pieces of gall tissue 3/4 to 1 1/4 inch in diameter. All chemicals were applied within 1 to 2 hours after collecting and preparing the test inoculum.

A U.C. soil mix (5) which contained by volume 50% Canadian peatmoss and 50% fine sand and fertilizer supplements was used. When the soil was fumigated the soil moisture was suitable for planting (approximately 30% water by weight).

The chemicals used were SMDC[3] (sodium N-methyl dithiocarbamate dihydrate), methyl bromide, and chloropicrin. Aqueous solutions of SMDC were applied with a sprinkling can to a 4-inch-deep layer of soil in flats until the soil was saturated. After draining, the flats were stacked for 7 days. Gaseous methyl bromide was applied for 24 hours to soil contained in stacks of flats which were enclosed beneath a polyethylene cover. Chloropicrin was applied with an hypodermic syringe into soil in glass jars (1 gallon) or cylindrical steel cans (30 x 14 inches diameter), and covered with a layer of wax paper beneath a tight fitting metal lid. After the containers were left sealed for 2 to 3 days, the soil was aerated for 5 days before retrieving the test pieces.

---

[1]Associate Plant Pathologist, University of California, Los Angeles.
[2]Plant Pathologist, Soil and Plant Laboratory, Orange, California. The authors wish to express their gratitude to Mr. O. A. Matkin, Director, Soil and Plant Laboratory, who graciously made the analyses.
[3][SMDC = Vapam.]

To evaluate the effectiveness of the chemicals, 3 to 6 pieces of infested tissue from each of two bags per treatment were retrieved, bulked, macerated and the infusion from the macerate used to inoculate 3 to 7 indicator plants. Tomato was used for A. tumefaciens and C. michiganense, and geranium was used for X. pelargonii. The plants were inoculated by the stem puncture method. Uninoculated controls were included with each experiment. In no case were the uninoculated controls infected, nor were the inoculated controls not infected.

For the nitrogen cycle studies a soil was prepared which contained negligible amounts of nitrate or ammonia nitrogen, as determined by prior analysis, to which was added an adequate supply of organic nitrogen (fine sand, Marina series, 1/2 bushel; Canadian peatmoss, 1/2 bushel; hoof and horn meal, 97.5 grams; treble super phosphate, coarse fraction, 97.5 grams; potassium sulfate, 32.5 grams; dolomite lime, 147 grams). The pH after mixing was 5.8. After steaming the test soil for 1 hour at 212° F, approximately 1/2 gallon of a greenhouse soil known to contain abundant nitrifying organisms was added to 1 bushel of the test soil. In this way the soil was supplied with ammonifying and nitrifying organisms, but the soil nitrogen initially was almost exclusively in the organic form. By following the changes in ammonia and nitrate nitrogen after treatment, a measure of the effects of the treatment on the nitrogen cycle organisms was obtained. One lot of soil was taken after mixing and steamed at 212° for 1 hour in cotton stoppered flasks. After 2 to 3 days the remainder of the soil was treated with the fungicides in the same manner as described for the bacterial survival tests, placed in sterile flasks, and stored at 74° in the dark. Nitrates were analyzed by the Brucine method and ammonia was analyzed by the Nessler method at periods of 60 to 70 days. Three experiments were made at intervals of several months.

RESULTS

Plant pathogenic bacteria: The data on the effects of three fungicides on plant pathogenic bacteria in soil are shown in Table 1. Some variation of lethal dose occurred between experiments as might be expected with data based upon the presence or absence of disease symptoms in inoculated test plants. However, by inspection of Table 1, a probable lethal dose may be postulated for each chemical-bacterium combination. This dose provides a starting point in

Table 1. Data on the effect of fungicides on plant pathogenic bacteria in host tissue in soil[a].

Treatment	Xanthomonas pelargonii				Corynebacterium michiganense				Agrobacterium tumefaciens			
	Experiment No.				Experiment No.				Experiment No.			
	1	2	3	4	1	2	3	4	1	2	3	4
Inoculated, untreated	+	+	+	+	+	+	+	+	+	+	+	+
Not inoculated, untreated	-	-	-	-	-	-	-	-	-	-	-	-
SMDC												
100 pounds/acre				+				+				+
200	+	+	+	+	+	+	+	+	-	+	+	+
300				∓				+				-
400	+	-	-	∓	+	+	+	+	-	-	-	-
800	∓	-	-	-	+	+	-	-	-	-	-	-
Probable lethal dose	400 pounds/acre				800 pounds/acre				300 pounds/acre			
Chloropicrin												
0.5 ml/cubic foot	+	+	+	+	-	-	+	+	-	+	-	+
1	-			∓	-			-	-	+		-
2		-	-	-		-	-	∓			-	-
4			-				-				-	
Probable lethal dose	2 ml/cubic foot				2 ml/cubic foot				1 ml/cubic foot			
Methyl bromide												
1 pound/100 cubic feet	+	+		+	+	+		+	+	+		+
2	+	-	-	+	∓	+	∓	+	-	-		+
4	-		-	-	-	-	-	-	-			-
Probable lethal dose	3 pounds/100 cubic feet				3 pounds/100 cubic feet				2 pounds/100 cubic feet			

[a] + All test plants diseased. ∓ More than half test plants diseased. - No test plant diseased. ∓ Less than half test plants diseased.

establishing commercial control recommendations for nursery crops, particularly in ground beds, raised benches, and containers.

There is so much variety in soils, crops, environment, and cultural conditions capable of influencing the lethal dose, that it seemed wise to terminate the trials at this point. Tests in greenhouse and field under more natural conditions will be needed before firm recommendations can be made for any given conditions.

The probable lethal dose is useful for comparisons within this study. It indicates that the three test species of bacteria responded similarly to treatment with methyl bromide and chloropicrin, but C. michiganense withstood twice the dose of SMDC that was lethal to X. pelargonii and nearly three times the dose lethal to A. tumefaciens. With exception of the reaction of C. michiganense and X. pelargonii to SMDC, all lethal doses were within the range commonly used to control fungus and nematode soil pathogens.

Soil nitrogen: The data on the effects of the chemical treatments on the soil nitrogen are summarized in Table 2. Since the untreated control had a very high proportion of nitrates to ammonia after incubating for 60 to 70 days, it indicated that the soil initially had high nitrifying capabilities. Treatment with chloropicrin, methyl bromide, and steam stopped nitrification, but more ammonifiers survived in the chemically treated soils than in the steamed soil. In the SMDC series a transition occurred from a dose that did not affect nitrification (100 pounds/acre), to one that partially inhibited it (200 pounds/acre), to higher doses that completely inhibited the process (400, 800 pounds/acre).

Table 2. Nitrate and ammonia nitrogen after 60 to 70 days in soil at 74° F treated as indicated.

Treatment	Nitrate nitrogen (ppm)				Ammonia nitrogen (ppm)			
	Experiment No.			Mean	Experiment No.			Mean
	1	2	3		1	2	3	
Not treated	275	224	125	208	5	88	8	34
Steamed	29	44	21	31	106	61	36	68
SMDC								
100 pounds/acre	-	-	302	302	-	-	39	39
200	224	-	24	124	74	-	226	150
400	21	-	17	19	210	-	143	177
800	6	-	16	11	167	-	118	143
Chloropicrin								
0.5 ml/cubic foot	25	38	-	32	164	208	-	186
1.0	24	39	-	32	149	217	-	183
Methyl bromide								
1 pound/100 cubic feet	22	11	-	17	133	52	-	93
2	15	40	-	28	130	194	-	162
4	19	38	-	29	133	160	-	147

The fact that the bacteria were killed in these tests relatively easily conflicted with some reports of others using different bacteria. Probably the reason for the differences is that in the cases of reported high resistance the inoculum was treated when very dry. With the cases reported here, as well as with those of McKeen (2), the treated material was in a fairly moist and active condition when fumigated. The potency of methyl bromide is very moisture dependent (6) and it seems probable that the moisture variations of the test substances may explain the divergent results reported in the literature.

From the results reported here, and from other studies, it seems probable that certain soil-borne plant pathogenic bacteria are as readily controlled by soil fungicides as are most soil fungus pathogens. Chemical control of such bacteria may be facilitated by allowing crop residue to rot several months prior to treatment.

Literature Cited

1. HALL, N. M., and L. F. L. CLEGG. 1949. Microbiological aspects of the partial sterilisation of soil by chemicals. Proc. Soc. Appl. Bact. 1949: 105-118.
2. McKEEN, C. D. 1954. Methyl bromide as a soil fumigant for controlling soil-borne pathogens and certain other organisms in vegetable seedbeds. Can. J. Botany 32: 101-115.

3. MacLACHLAN, D. S., et al. 1953. Fumigation of used bags with toxic gases for the control of bacterial ring rot of potato. Can. J. Agr. Sci. 33: 132-140.
4. MAI, W. F., BERT LEAR, and C. W. BOOTHROYD. 1951. Ineffectiveness of methyl bromide for killing the causal organisms of ring rot and seed piece decay of potatoes. Plant Disease Reptr. 35: 356-357.
5. MATKIN, O. A., and P. A. CHANDLER. 1957. The U.C.-type soil mixes. Sec. 5. California Agr. Exp. Sta. Manual 23: 68-76.
6. MUNNECKE, D. E., R. A. LUDWIG, and R. E. SAMPSON. 1959. The fungicidal activity of methyl bromide. Can. J. Botany 37: 51-58.
7. RICHARDSON, L. T. 1957. Quantitative determination of viability of potato ring rot bacteria following storage, heat and gas treatments. Can. J. Botany 35: 647-656.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA

WATERMELON DISEASE INCIDENCE IN CENTRAL FLORIDA, 1931-1959[1]

N. C. Schenck[2]

Abstract

The three predominant foliage diseases of watermelon in Florida from 1931-1959 were downy mildew (Pseudoperonospora cubensis), anthracnose (Colletotrichum lagenarium), and gummy stem blight (Mycosphaerella citrullina). Abundant occurrence of downy mildew was correlated with above-normal rainfall in May and June following one or more years of mild winter temperatures. Abundant occurrence of anthracnose was correleated with above-normal rainfall in June following spring seasons with above-normal rainfall throughout most of the period. Abundant occurrence of gummy stem blight was correlated with above-normal rainfall during the period from February through May. Widespread use of resistant varieties since 1955 has reduced losses from Fusarium wilt and has increased the relative importance of foliar diseases in watermelon production in Florida.

Since 1931 watermelon disease occurrence in Florida has been observed by members of the staff of the Watermelon and Grape Investigations Laboratory of the Florida Agricultural Experiment Stations. The purpose of this report is to summarize these observations and relate them to weather data for this same period.

Information on disease occurrence was obtained from the Annual Reports, bulletins, unpublished progress reports and other publications by the staff of this Station. Weather data were obtained from Warren O. Johnson, Weather Bureau, United States Department of Commerce, Lakeland Florida and Florida Agricultural Experiment Stations.

LEAF DISEASES OF GENERAL OCCURRENCE

Anthracnose (Colletotrichum lagenarium (Pass.) Ell. & Halst.).
This disease affects leaves, stems and fruits in the field or in transit and for years was considered the most serious disease of watermelon in Florida. However, in recent years anthracnose has become of lesser importance mainly because of widespread use of resistant varieties, universal seed treatment by seed producers, and common usage by growers of fungicidal sprays such as maneb and zineb[3]. There has been no appreciable increase in the incidence of anthracnose in recent years, despite the fact that more virulent physiologic strains of C. lagenarium have been described (2). Anthracnose has not been recorded as occurring in abundance in Florida since 1942 (Fig. 1).

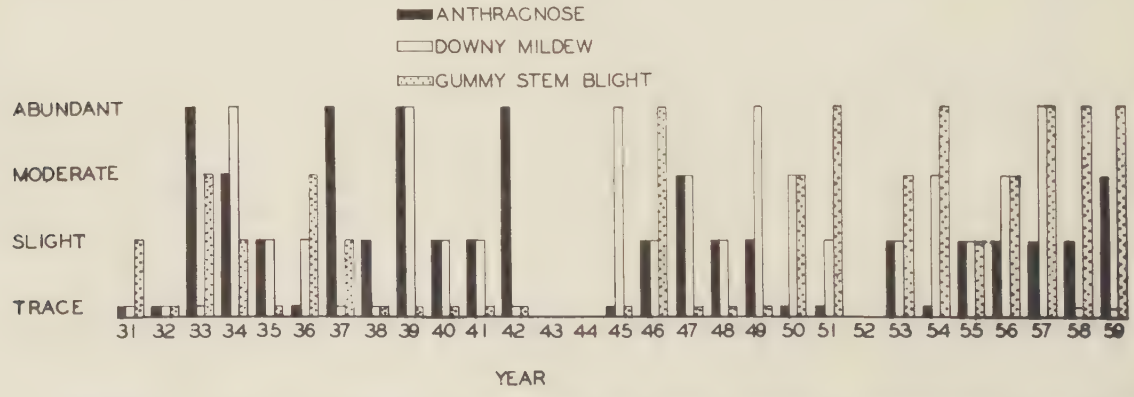

ANTHRACNOSE
DOWNY MILDEW
GUMMY STEM BLIGHT
ABUNDANT
MODERATE
SLIGHT
TRACE
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59
YEAR

FIGURE 1. Yearly incidence of three major leaf spot diseases of watermelon, 1931-1959. (No records of disease occurrence are available for years 1943, 1944, and 1952.)

[1] Florida Agricultural Experiment Station Journal Series, No. 1086.

[2] Assistant Plant Pathologist, Watermelon and Grape Investigations Laboratory, Florida Agricultural Experiment Stations, Leesburg, Florida.

[3] maneb = manganous ethylene bisdithiocarbamate; zineb = zinc ethylene bisdithiocarbamate.

Downy mildew (Pseudoperonospora cubensis (Berk. & Curt.) Rostow.).
This disease has occurred in Florida in abundance 5 of the years from 1931-1959 (Fig. 1). However, it is potentially a serious disease every year because of its characteristic rapid spread (by wind dissemination) and almost perpetual existence in south Florida on wild or cultivated cucurbits. With the lessened incidence of anthracnose in Florida, downy mildew is the most serious late season defoliator of watermelons in some years. Control is difficult since none of the present commercial varieties are resistant and practical field control can be obtained only by well-timed and well-executed applications of fungicides.

Gummy stem blight (Mycosphaerella citrullina (C. O. Sm.) Gross.).
This disease has occurred commonly for many years as a crown and leaf infection. Recently the fruit rot phase of the disease has become more extensive, resulting in the rejection at terminal markets of carload lots of Florida watermelons. The increased incidence of this fruit rot has been coincidental with the widespread use of the variety Charleston Gray. At present gummy stem blight is the most serious early season disease of watermelon. The usual seed treatments, tetrachloroquinone (chloranil) or tetramethyl thiuram disulfide (thiram) do not disinfect seed of the causal organism. There are no resistant varieties and field control is difficult to obtain with fungicides presently available.

Cercospora leaf spot (Cercospora citrullina Cke.).
This has been considered a minor foliar disease in Florida. In 1954, when most other diseases were slight in occurrence, it was one of the major defoliators. Increased incidence of the disease has been coincidental with common usage of the variety Charleston Gray. Cercospora leaf spot has been relatively easy to control with several fungicides.

VASCULAR DISEASES OF GENERAL OCCURRENCE

Fusarium wilt (Fusarium oxysporum (Schlecht.) f. niveum (E. F. Sm.) Snyder & Hansen).
The incidence of this disease varies slightly from year to year. Fusarium wilt is usually serious every year with non-resistant varieties grown on land planted previously (5 or less years) in watermelons. The disease has not been severe in the past 5 years because of common grower usage of resistant varieties and the predominant use of "new" land.

DISEASES OF MINOR OCCURRENCE

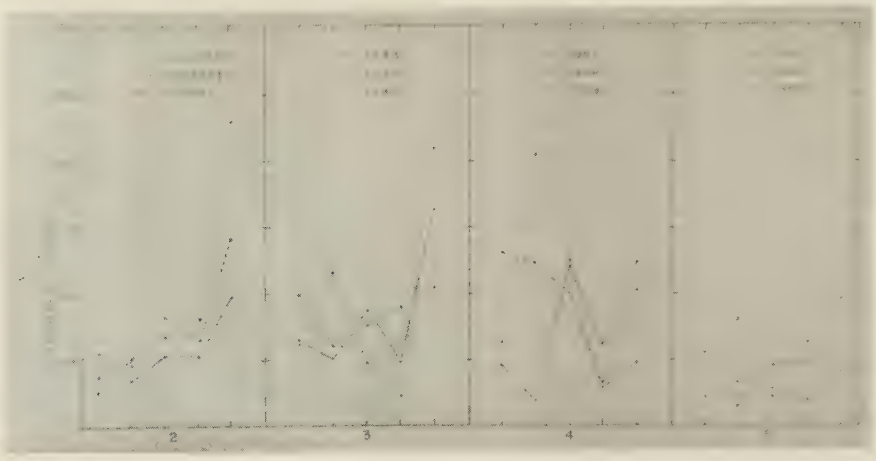
Alternaria leaf spot (Alternaria cucumerina (Ell. & Ev.) J. A. Elliott) was of slight and infrequent occurrence except for 1934, 1936, and 1957, when it occurred in moderate amounts. Powdery mildew (Erysiphe cichoracearum DC.) occurred rarely. Although watermelon mosaic has been reported infrequently, it usually occurred extensively in areas from which it was reported. Root-knot nematode (Meloidogyne spp.) was recorded infrequently and usually from fields previously planted in watermelon.

RELATION OF DISEASE OCCURRENCE TO WEATHER

Since disease incidence reports were frequently vague or general in nature, detailed correlations between rainfall, temperature or other weather data and disease occurrence were not attempted. However, there were noteworthy correlative trends between total monthly rainfall and disease incidence.

The abundant occurrence of downy mildew, a late season disease, was correlated with above-normal rainfall in May and June, particularly the latter, following one or more years of mild winter temperatures. In the 5 years (1934, 1939, 1945, 1949, 1957) that downy mildew occurred abundantly (Fig. 1), total rainfall during May and June measured 21.5, 20.5, 16.6, 10.5, and 10.6 inches, respectively, compared with a 57-year mean rainfall for this period of 9.2 inches (1). In the 4 years (1947, 1950, 1954, 1956) that downy mildew occurred moderately, total rainfall during May and June measured 14.4, 8.7, 9.4, and 9.1 inches, respectively. In 17 years that downy mildew occurred slightly or in trace amounts, 6 had May-June rainfall considerably below the mean rainfall for the period and 6 (with rainfall normal or above) were preceded by unusually severe winters (3). Failure of downy mildew to develop in years following severe winters is attributed to the winter-killing of wild and cultivated hosts of P. cubensis in those years. Mean monthly rainfall during the period February-June for years of abundant, moderate, and slight downy mildew occurrence is shown in Figure 2.

Abundant or moderate occurrence of anthracnose, predominantly prior to the introduction of the resistant variety Congo in 1948, was correlated with above-normal rainfall in June following spring seasons (March, April, May) with normal or above-normal rainfall throughout



FIGURES 2-5. 2 -- Mean monthly rainfall February-June for years of slight, moderate and abundant downy mildew occurrence.
3 -- Monthly rainfall February-June for 3 years of abundant anthracnose occurrence.
4 -- Monthly rainfall February-June for 3 years of abundant gummy stem blight occurrence.
5 -- Monthly rainfall February-June for 3 years of slight foliar disease occurrence.

most of the period. In 6 years (1933, 1934, 1937, 1939, 1942, 1947) that anthracnose occurred moderately or abundantly (Fig. 1), June rainfall measured 6.2, 13.4, 5.2, 15.6, 10.6, 11.6 inches, respectively, compared with a 57-year mean rainfall for June of 6.3 inches (1). In all these years, rainfall during the period March through May was above-normal and rather evenly distributed. In 3 of 9 slight or trace anthracnose years, June rainfall was considerably below normal. In 4 of the other 6 years, rainfall during March-May was below normal or its distribution was such that dry periods ensued. Typical rainfall patterns for seasons of abundant and slight anthracnose occurrence are shown in Figures 3 and 5.

Abundant or moderate occurrence of gummy stem blight, predominantly after 1946, was correlated with normal or above-normal rainfall during the period from February through May. In the 6 years (1946, 1951, 1954, 1957, 1958, 1959) that gummy stem blight occurred abundantly (Fig. 1) total rainfall for the period measured 9.2, 10.7, 10.2, 16.0, 19.7, and 22.9 inches, respectively, compared with a 57-year mean rainfall for this period of 10.6 inches. In the 3 years (1950, 1953, 1956) that gummy stem blight occurred moderately, total rainfall for this period measured 11.6, 14.0, and 8.8 inches, respectively. In 3 of 4 years (1948, 1949, 1955) that gummy stem blight occurred in only slight amounts, rainfall for the February-May period was well below the average for the period (7.9, 6.3, and 7.2 inches, respectively). Failure of gummy stem blight to develop in 1947 could not be explained by shortage of rainfall. Typical rainfall patterns for seasons of abundant and slight gummy stem blight occurrence are shown in Figures 4 and 5.

Literature Cited

1. ANONYMOUS. Meteorological data for Orlando, Florida. Weather Bureau, U. S. Dept. of Commerce.
2. GOODE, MONROE J. 1958. Physiological specialization in Colletotrichum lagenarium. Phytopathology 48: 79-83.
3. JOHNSON, WARREN O. 1958. Winter minimum temperature in peninsular Florida. Weather Bureau, U. S. Dept. of Commerce, and University of Florida Agr. Exp. Sta. 70 pp.

FLORIDA AGRICULTURAL EXPERIMENT STATION, LEESBURG, FLORIDA

TOXICITY OF PCNB TO MAGNOLIA FUSCATA

Natale Zummo and A. G. Plakidas

During the course of a spray program for the control of petal blight of camellia, using pentachloronitrobenzene 75% WP (PCNB=Terraclor) as a ground spray, it was noticed that Magnolia fuscata plants in the vicinity were defoliated. The fallen leaves and isolated leaves that remained on plants presented a very striking appearance. The leaves became chlorotic, but not uniformly; numerous circular islands of dark-green color, ranging in size from pin-point to about 5 mm, remained on the bleached background (Fig. 1).

FIGURE 1. Typical injury to Magnolia fuscata, consisting of chlorotic leaves with scattered dark-green spots.

The first case that came under observation occurred in a commercial nursery. The ground under and around a group of camellias was sprayed with Terraclor at the rate of 200 pounds per acre the latter part of December 1959. A group (over 1000) of 3-year-old Magnolia fuscata plants growing in containers (gallon cans), and situated at distances of from 22 to 40 feet from the sprayed ground, developed the striking spotting in their foliage and became practically completely defoliated within 2 to 3 weeks after the ground application of Terraclor.

The second case occurred in a private garden in another locality. Approximately 3 weeks after the ground of this garden was sprayed with Terraclor, at the same rate as in the previous case, two large M. fuscata trees (about 20 feet in height) situated at the entrance of the garden showed the same pattern of leaf symptoms and gradually dropped most of their leaves.

In the first case injury evidently resulted from spray drift getting directly on the foliage. The plants were growing in containers, not in direct contact with the sprayed ground, and the Terraclor could not have been taken up through their roots. However, the situation was not so clear in the second case. There the ground under the two large M. fuscata trees was sprayed with Terraclor, and the injury to the foliage could have been caused either by spray drift or by absorption of the chemical by the roots of the trees. The following tests were undertaken in an attempt to obtain more definite information on this point.

Three large (4 to 5 feet in height) M. fuscata plants growing outdoors were sprayed on January 25 with Terraclor at the rate of 10 g per gallon of water until runoff occurred. The soil under three similar plants was treated with Terraclor as a soil drench, using the chemical at the rate of 200 pounds per acre. Care was taken to avoid getting any of the material on the foliage. Similar untreated plants were retained as a control.

By February 1 the leaves of the sprayed plants became spotted and started to drop; by February 15 the plants were completely defoliated. In the plants in which the Terraclor was applied to the soil, spotting and leaf shedding appeared on the lower branches only by February 1. Leaf spotting and leaf dropping progressed upward gradually and slowly, and in about 1 month the topmost branches of the plants were affected. The control plants remained normal.

The results of this test showed definitely that Terraclor is injurious to M. fuscata when applied as a spray to the foliage, and seemed to indicate also that the plant could take up the chemical from the soil through the roots. However, since a hard rain occurred the second day after the chemical was applied, and since the spotting and defoliation on the plants growing in treated soil started on the lower leaves, the spotting may have resulted from splashing of the chemical onto the lower leaves by the rain. Therefore, in another test the soil under two other plants was treated with the chemical on February 23 and immediately covered with heavy mulch (8 inches of pine straw) to prevent splashing of the chemical on the plants. By March 22 spotting and defoliation was evident on the lower leaves, and it advanced gradually upwards.

The injury resulting from spray drift appears to be transient. The container-grown plants that were completely defoliated have recovered and produced new foliage that appears perfectly normal. It is too early to tell whether soil application of the chemical will cause chronic injury.

BOTANY DEPARTMENT, LOUISIANA STATE UNIVERSITY, BATON ROUGE, LOUISIANA

EVALUATION OF PHALTAN FOR THE CONTROL OF GRAPE DISEASES[1]

E. F. Taschenberg and A. J. Braun

Summary

Phaltan has given effective control of powdery mildew on Concord grapes in New York. In limited field tests it has also given control of black rot and downy mildew. No phytotoxicity was observed in the Phaltan-sprayed plots in 1957 or 1958. However, in 1959 foliage injury was observed in all of the Phaltan-sprayed plots. Phaltan deposits were removed from grapes rather rapidly by weathering. Losses, on a weight basis, were 60% or more 28 days after the final spray application. The Phaltan residue on fresh grapes 14 days after the final application was 8.6 ppm, while juice extracted from these grapes contained less than 0.2 ppm of Phaltan. The Phaltan residue on grapes sampled 49 days after the final application was 2.3 ppm, while less than 0.1 ppm was found in the extracted juice. Three Phaltan-DDT sprays had no consistent effect on fruit quality as measured by percentage of soluble solids.

INTRODUCTION

Powdery mildew (Uncinula necator (Schw.) Burr.) and black rot (Guignardia bidwellii (Ell.) Viala & Ravaz) are frequently serious on the fruit of Concord grapes in New York. Although downy mildew (Plasmopara viticola (Berk. & Curt.) Berl. & de T.) is seldom found on the Concord variety, it frequently causes serious damage to the fruit or foliage of certain other varieties (4).

For disease control where black rot is not likely to be serious, a three-spray schedule is currently recommended: ferbam immediately after bloom and low-soluble copper and lime 7 to 10 days later and again in August. Where additional black rot protection is needed, a before bloom spray of ferbam is suggested (1). The post bloom sprays include an insecticide for control of grape berry moth (Paralobesia viteana Clem.). Although timing of the August application is contingent upon grape berry moth activity, disease control with these schedules has been satisfactory.

There are several objections to the presently recommended schedule. Ferbam, although highly effective for control of black rot, does not control powdery mildew (1, 4). Low-soluble copper with lime, although very effective for control of powdery mildew and downy mildew, is much less effective than ferbam for the control of black rot. The incompatibility of these fungicides makes it necessary to apply them separately. Lime, which must be added to the low-soluble copper fungicides, decreases the effectiveness of Sevin and possibly certain other insecticides. Furthermore, the amount of lime required in concentrate sprays is likely to plug the nozzles.

The objectives of this study were to compare Phaltan with the presently recommended fungicides for grape disease control, to determine the Phaltan residues on fruit and in juice, and to determine the effect of the fungicide on soluble solids.

MATERIALS AND METHODS

The fungicides used were ferbam (50% ferric dimethyldithiocarbamate); COCS (basic copper chloride, basic copper sulfate); Orthocide (50% N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide); and Phaltan (50% N-trichloromethylthiophthalimide), an analog of Orthocide. Several spreaders and spreader-stickers were used. The "lime" referred to in the tests was hydrated spray lime with a high magnesium content. The concentrations of the fungicides and supplements used in the sprays are given in the footnotes for Table 1. The ferbam and COCS sprays contained DDT (50% w.p.), 1.5 pounds per 100 gallons. Phaltan sprays contained either DDT or Sevin (50% naphthyl N-methyl carbamate) at 1.5 and 2 pounds, respectively, per 100 gallons.

The treatments were applied with a hooded boom equipped with 14 nozzles (5). For the first and second sprays the rate of application was 250 gallons per acre and for the third 300 gallons per acre. All plots were at least four rows wide and 12 or more vines long.

[1]Approved by the Director as Journal Paper No. 1219, New York State Agricultural Experiment Station, Geneva, New York.

Disease control data were taken during the week prior to harvest. The incidence of powdery mildew was determined by recording the number of clusters having more than a trace of powdery mildew, based on the examination of 100 clusters per replicate in each treatment. Black rot data were obtained by recording the percentage of rotted berries on each of 100 clusters in each replicate; each cluster rated according to the following graded scale: 0, 1-9, 10-24, 25-49, 50-74, 75-89, 90-99, 100%. The severity of downy mildew was determined by estimating the percentage of foliage affected by downy mildew on each of 40 vines in each replicate, using the graded scale just defined.

Samples of fresh fruit for residue analysis were taken according to the procedure described by Taschenberg and Avens (6). The deposit was removed from the berries by rotating the sample for 20 minutes in 500 cc of benzene. The analyses[2] were made according to the method used for determining captan residues (7). Since the number of berries in all samples was the same, losses owing to weathering can be readily determined from the data. As to the effect of growth on deposit, it is calculated from the equation: B-A ÷ B x 100 = percentage loss by growth, where A is equal to weight of berries in initial sample and B is equal to weight of berries at date of sampling.

Juice samples were prepared from a 30-pound composite of fruit picked from 10 vines. A sample of 300 berries, 5 berries from each of 60 clusters selected at random, was taken for determining residue on fruit prior to processing. In preparing juice the procedure followed was similar to that employed in commercial processing. Phaltan was removed from the juice by extracting with benzene for 5 minutes. The sample was centrifuged for 10 minutes at 2000 rpm to separate the benzene from the juice. The method of analysis was the same as that used for fresh fruit.

Fruit samples for soluble solids determinations were taken from lightly pruned and from severely pruned vines on which the number of buds left per vine was proportional to the weight of 1-year cane prunings (3). Samples were taken from vines of comparable vigor, as determined by the weight of 1-year prunings, from four plots of each treatment at the beginning of harvest. This sampling was limited to two vines per plot in 1957 and to three vines per plot in 1958 and 1959. Only the apical four or five berries from the basal clusters were sampled during 1957 and 1958 (2). In 1959 one apical berry was taken from each basal cluster on shoots arising from the fourth bud and beyond on the canes. The percentage soluble solids was determined with a hand refractometer.

RESULTS AND DISCUSSION

Disease Control: Excellent control of powdery mildew resulted in seven of the eight tests in which Phaltan was used in the second and third post bloom applications (Table 1). The addition of Ortho sticker (ML-104) in combination with Ortho Spreader-Sticker appeared to improve disease control with Phaltan in 1958 in test 2 and especially in test 3 where the vines were considerably less vigorous with many clusters exposed to weathering. Powdery mildew control in the plots sprayed with ferbam or Orthocide in the first or second post bloom applications cannot be attributed to these fungicides inasmuch as they are known to be ineffective against this disease. COCS in the second and third post bloom applications gave excellent control of powdery mildew. As a single application following two ferbam sprays, Phaltan was less effective than COCS for the control of powdery mildew.

In 1958 Phaltan 2-100 applied immediately after bloom, 7 to 10 days later, and in early August controlled black rot on Concord grapes as well as one application of ferbam 1 1/2-100 followed by two applications of COCS and lime. In this test 18.4% of the berries were rotted in the check plots while 0.7 and 0.2% were rotted in the Phaltan and ferbam-COCS plots, respectively.

On the Delaware variety, downy mildew affected 40.8% of the foliage of unsprayed vines and less than 1.0% on the vines sprayed with Phaltan, Orthocide or ferbam followed by COCS. In this test an additional application was made immediately before bloom.

Spray Injury: Light to moderate injury was reported from Missouri by H. G. Swarthout in 1957[3]. This type of injury was not observed on Phaltan-DDT sprayed vines in New York in

---

[2]The authors are indebted to Mr. S. D. Gibbs of the Department of Food Science and Technology for the Phaltan analyses.

[3]Personal communications from H. G. Swarthout, Professor of Horticulture, University of Missouri, Columbia, Missouri.

Table 1. Powdery mildew control on Concord grapes with sprays of Phaltan and COCS.

Treatment[a] Post-bloom applications[b]			Supplement in sprays[c]	% clusters with more than a trace of powdery mildew										
				1957 test	1958 tests						1959 tests			
1st	2nd	3rd		1	1	2	3	4	5	6	1	2	3	4
O	O	O	O	96	97	96	100	62	99	100	100	92	100	92
P	P	P	A	--	--	0	10	1	--	--	0	0	--	1
P	P	P	B	0	3	5	43	--	--	--	--	--	--	--
C	P	P	C	--	--	--	--	2	--	--	--	--	--	1
F	Cu	Cu	D	0	3	--	0	3	--	--	0	0	2	1
F	F	O	B	--	--	--	--	--	97	98	--	--	--	--
F	F	P	B	--	--	--	--	--	38	51	--	--	--	--
F	F	Cu	D	--	--	--	--	--	13	21	--	--	--	--

[a]Fungicide and amount per 100 gallons: P = Ortho Phaltan (50% w.p.), 2 pounds; C = Orthocide (50% w.p.), 2 pounds; F = ferbam (50% w.p.), 1 1/2 pounds; Cu = COCS, 2 pounds, plus lime, 4 pounds.

[b]First = immediately after bloom; second = 7 to 10 days after bloom; and third = second brood berry moth application (late August).

[c]Supplement and amount per 100 gallons: A = Ortho Sticker (ML-104), 8 ounces, in all 1958 sprays, plus Ortho Spreader-Sticker, 4 ounces, in third 1958 spray; Ortho Spreader-Sticker, 4 ounces, in all 1959 sprays; B = DuPont Spreader-Sticker, 4 ounces, in all sprays; C = Ortho Sticker (ML-104), 8 ounces, in all 1958 sprays; Ortho Spreader-Sticker, 4 ounces, in all 1959 sprays; D = DuPont Spreader-Sticker, 4 ounces, or emulsible oil (Triton B1956, 1 part; Sovaspray Oil No. 3, 9 parts), 1 quart, in all sprays.

Table 2. Comparison of actual and apparent losses of Phaltan deposit after final spray, 1958 and 1959.

Time after last application (days)	Phaltan deposit By weight ppm[a]	Grams	% reduction[b]	Per 100-berry sample Micrograms	% reduction[c]	% reduction due to growth	Accumulated rainfall (inches)
	Phaltan (50% w.p.) 1.5 pounds[d] Applied 6/19, 6/29, and 8/10, 1959						
0	11.6	223	0	2587	0	0	0.00
14	5.9	241	49	1422	45	8	0.41
28	4.4	263	62	1157	55	15	1.06
	Phaltan (50% w.p.) 2 pounds[e] Applied 7/4, 7/17, and 8/26, 1958						
0	24.7	218	0	5385	0	0	0.00
7	18.9	240	24	4536	16	9	0.28
14	12.0	264	52	3168	41	17	3.11[g]
28	6.9	283	72	1953	64	23	5.69
	Applied 6/19, 6/29, and 8/10, 1959[f]						
0	14.2	230	0	3266	0	0	0.00
3	12.2	233	14	2843	13	1	0.00
7	9.0	219	37	1965	40	0	0.28
14	6.9	255	51	1759	46	10	0.41
21	7.2	262	49	1886	42	12	0.96
28	5.6	261	60	1462	55	12	1.06

[a]Net values after deduction of value on untreated fruit in 1958 3.9, 3.6, 1.4, 1.9 ppm respectively at 0, 7, 14 and 28 days, and 0.0 ppm in 1959. Recovery of known amounts added to fruit 100% in 1958 and 99% in 1959.

[b]Apparent loss.

[c]Actual loss.

[d]DuPont Spreader-Sticker, 4 ounces.

[e]Ortho Sticker (ML-104), 8 ounces, in applications 1, 2, and 3; Ortho Spreader-Sticker.

[f]Ortho Spreader-Sticker, 4 ounces.

[g]Rainfall between 9/2 and 9/9, 2.94 inches, 1.06 inches in 8 hours on 9/7 and 1.21 inches in 7 hours on 9/8.

1957 or 1958. In 1959, however, slight to moderate foliage injury was observed in all plots treated with Phaltan-DDT sprays. The injury, which was evident by late July, became more severe and was most pronounced in mid-September.

The injury was characterized by large areas of light green which usually appeared first near the center of the leaf. As these areas enlarged they became yellow and eventually necrotic near the center. Severely affected leaves dehisced. On east-west rows the south side of the row showed severe foliage injury whereas on the north side little or no injury occurred. On north-south rows the west side showed more injury than the east side. Injury was most severe where drought conditions prevailed. Under these conditions premature dropping of foliage was not uncommon.

At Fredonia, where severe injury was observed, there were 13 days between July 1 and September 30 on which the temperature was 90° F or higher in 1959 as compared with 0 days in 1958 and only 1 day in 1957. Rainfall from June 1 through September 30 was 6.17 inches below normal in 1959, whereas it was 6.24 inches above normal in 1958 and about normal in 1957. These observations suggest that the development of Phaltan injury is dependent upon high temperatures, low soil moisture, and very low rainfall. In view of Phaltan's effectiveness for the control of black rot, downy mildew, and powdery mildew, additional studies to evaluate its effectiveness at lower concentrations and to determine means of decreasing its phytotoxicity are warranted.

Phaltan Deposits and Residues: In 1958 a Phaltan deposit of 24.7 ppm was found on the fruit immediately after the final spray application was made (Table 2). Approximately 30% of this original deposit was present 4 weeks later. Growth accounted for a "loss" of 23% while the actual loss was 64%. There was no appreciable difference in deposition or loss of Phaltan residues with the various spray supplements.

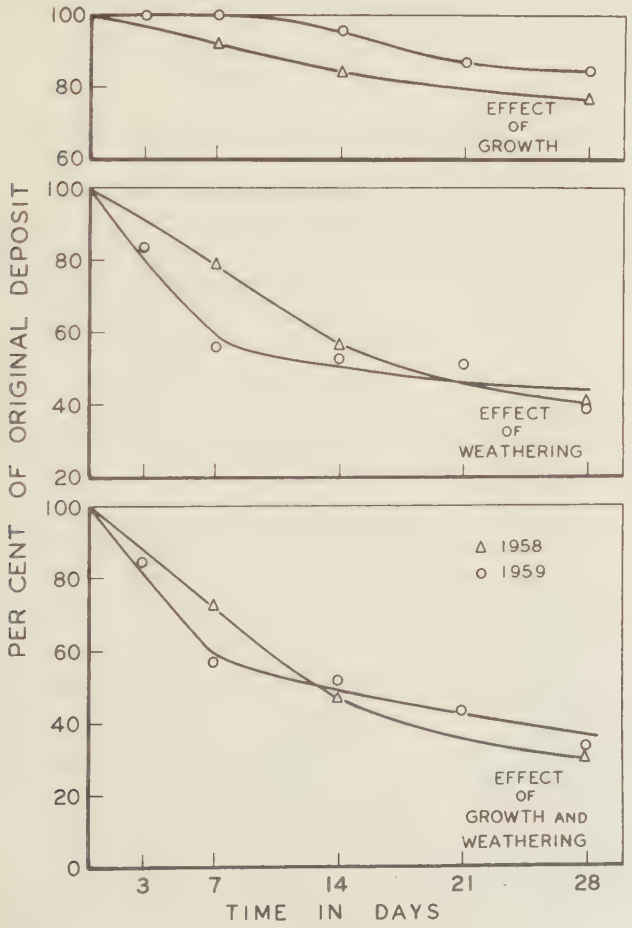


FIGURE 1. Separate and combined effects of growth and weathering on Phaltan deposits, 1958 and 1959. Phaltan (50% w.p.), 2 pounds; DDT (50% w.p.), 1.5 pounds; and DuPont Spreader-Sticker, 4 ounces, in 100 gallons of water.

The initial deposits in comparable tests (2-100 rates) were lighter in 1959 than in 1958, namely, 14.2 and 24.7 ppm (Table 2). Growth was less important than weathering in reducing Phaltan residues (Fig. 1). Weathering caused a loss of from 40 to 44% within 1 week in 1959. In the preceding season 2 weeks were required to effect a similar loss. Undoubtedly the high temperature was chiefly responsible for the rapid loss in Phaltan deposits recorded in 1959. Temperatures and rainfall in the 4-week period following the cessation of spraying were quite different for these 2 years. In 1959 the maximum temperature was 85° F or higher on 20 of the 28 days while on 7 of these days it was 90° or higher. The temperature was 85° or higher on only 3 days during this period in 1958. The rainfall during the 28-day post-spray period was 1.06 inches in 1959 and 5.69 inches in 1958.

Relatively small amounts of Phaltan were found in juice pressed from Phaltan-sprayed grapes. The Phaltan residue present in the juice was less than 2% of that on the fresh fruit. This disappearance may be attributed to decomposition of Phaltan as a result of the high temperatures to which the fruit is subjected before pressing (160° F) and during flash pasteurization (180°) and/or a possible tendency of the residue to remain on the skins, which are eliminated with the pomace.

Table 3. Phaltan residues on fresh grapes and in juice, 1958.

Treatment[a] (in pounds)	Date of application	Days between final application and sampling	Phaltan residue (ppm)			
			Fresh[b] grapes	Juice[c]		
				Rep. A	Rep. B	Average
Phaltan, 2	7/4, 7/17, 8/27	49	2.3	<0.1	<0.1	<0.1
Phaltan, 2	7/4, 7/17, 8/27, 10/1	14	8.6	0.1	0.2	0.1

[a]Sevin (50% w.p.), 2 pounds; DuPont Spreader-Sticker.
[b]Net residue after deducting 3.2 ppm blank value. Recovery of known amounts added to unsprayed fruit, 100%.
[c]Recovery of known amounts, 107%; values not corrected. Net values after deduction of value on juice made from untreated fruit, 0.31 ppm.

Table 4. Effect of various fungicide-DDT schedules on soluble solids content of Concord grapes.

Year	Treatment[a] Post bloom applications[b]			Pruning severity[c]	Average weight per vine[d] (in pounds)		% soluble solids[e]
	First	Second	Third		1-year prunings	Fruit	
1957	O	O	O	Light	1.2	14.9	16.2
				Severe	1.1	12.9	16.0
	P	P	P	Light	0.9	11.4	17.3
				Severe	1.0	10.5	17.2
	F	Cu	Cu	Light	1.2	13.9	16.8
				Severe	1.0	10.0	16.1
1958	O	O	O	Light	1.3	18.0	14.5
				Severe	1.4	14.1	14.6
	P	P	P	Light	1.6	16.6	14.6
				Severe	1.5	13.7	14.8
	F	Cu	Cu	Light	1.4	17.3	14.9
				Severe	1.5	13.8	14.6
1959	O	O	O	Light	1.7	19.9	14.9
				Severe	1.8	19.0	14.9
	P	P	P	Light	1.6	18.8	14.0
				Severe	1.8	16.4	14.7
	F	Cu	Cu	Light	1.6	19.2	15.1
				Severe	1.8	16.9	15.8

[a, b]See footnotes for Table 1. Supplement B used with Phaltan; D used with ferbam-COCS schedule (see footnote c for Table 1).
[c]Light pruning (40 + 10 buds). Severe pruning (20 + 20 buds).
[d]Production on vines sampled.

Quality of Fruit: One of the more important measurements of fruit quality is soluble solids. Thus, the effect that spray treatments might have on soluble solids content is important. The soluble solids content of fruit from vines receiving three Phaltan-DDT applications was approximately 1% higher than that of fruit from unsprayed vines in 1959; approximately the same in 1958; and somewhat lower in 1959 (Table 4). The ferbam-COCS-DDT spray schedule had no appreciable effect on the soluble solids content. It should be noted that these results were obtained on vines having somewhat below average vigor.

Literature Cited

1. BRAUN, A. J. 1948. Fungicides for the control of grape diseases. Farm Research 14(2): 12.
2. SHAULIS, N. J. 1956. The sampling of small fruits for composition and nutritional studies. Proc. Am. Soc. Hort. Sci. 68: 576-585.
3. SHAULIS, N. J., and G. D. OBERLE. 1948. Some effects of pruning severity and training on Fredonia and Concord grapes. Proc. Am. Soc. Hort. Sci. 51: 263-270.
4. SUIT, R. F. 1945. Field results on the control of certain grape diseases in New York. New York State Agr. Exp. Sta. (Geneva) Bull. 712.
5. TASCHENBERG, E. F. 1948. Hooded booms for grape spraying. New York State Agr. Exp. Sta. (Geneva) Bull. 732.
6. TASCHENBERG, E. F., and A. W. AVENS. 1960. DDT deposits on grapes as affected by growth and weathering. J. Econ. Ent. (In press)
7. WAGNER, JUANITA, VOLNEY WALLACE, and JOHN M. LAWRENCE. 1956. Determination of captan. J. Agr. and Food Chem. 4: 1035-1038.

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION, GENEVA, NEW YORK

TRUNK AND BRANCH CANKER OF COFFEE TREES IN GUATEMALA[1]

William C. Snyder, E. E. Trujillo, K. Cameron McOnie, and Carlos A. Berger[2]

The coffee canker or "Llaga macana" of South America, caused by Ceratocystis fimbriata (Ell. & Halst.) Hunt has been found prevalent in the western coffee-growing areas of Guatemala at 2000- to 4500-foot elevations. The disease is a limiting factor in coffee production in some fincas, causing serious losses in the more humid, upland plantings.

The Ceratocystis (Ceratostomella) canker of coffee was reported first by Pontis (4) from Venezuela and Colombia in 1951, by Szkolnik (5) from a Guatemala coffee finca in the same year, and by Echandi (1) from Costa Rica in 1955. Earlier and recent reports in the literature describe Nectria sp. as the causative agent of a similar disease on coffee in Guatemala (2,3).

In February 1959, Ceratocystis sp. and Nectria sp. (Imp. Fusarium solani) were isolated from canker material collected in Guatemala. In May of the same year single spore cultures of the two organisms were taken back to Guatemala and inoculated into young (1-year-old nursery trees) and old coffee plantings of the varieties "Arabica" and "Burbon" to determine with certainty the cause of the Guatemala canker.

Pure cultures of these fungi were introduced separately and in combination in small 3/8-inch cuts and held in place on the plant by plastic tape. For checks, plants were wounded but not inoculated. The plants were inoculated at the base of the stem (6 inches from the soil line) and on branches above. The plantings were located at elevations of 2600 feet, 3200 feet, and 4200 feet. Two hundred and fifty inoculations were made at the three locations.

The canker inoculation experiments have shown rather conclusively that Ceratocystis is the cause of the coffee canker prevalent in western Guatemala and that Nectria is a saprophyte. Ceratocystis cankers appear to be associated with machete wounds. This was observed in many naturally infected trees, indicating that the fungus is a wound pathogen. All the plants inoculated with Ceratocystis, either alone or in combination with Nectria, exhibited the typical canker of the disease, characterized by blackening and killing of the cortex, phloem, cambium, and xylem tissues. All plants inoculated with Nectria alone healed normally, as did all of the check trees. Nectria was found fruiting on dead bark and other parts of plants which had died from other causes. The typical red perithecia of this fungus were observed very abundantly on the bark of inoculated trees as well as on non-inoculated ones.

After 10 months 75% of the nursery trees inoculated with Ceratocystis were dead while the remainder were girdled. None of the old trees was killed at the end of 10 months. The rate of canker enlargement is indicated by the following measurements: average of five stem bases inoculated June 2, 1959 was 2 inches long (longitudinal axis) and 0.75 inches wide by July 22, 1959; the same lesions averaged 4.6 inches in length and 1.38 inches wide by September 9, 1959, and 10.5 inches in length and 4.5 inches wide by March 25, 1960. The measurements on canker enlargement seem to indicate that the fungus grows faster along the vertical axis than the lateral axis of the plant. The rate of growth along the vertical axis averaged about 1 inch per month and less than 1/2 inch per month along the lateral axis. Stem-base inoculations show that the fungus grows downward at a much more rapid rate than in the upward direction. In more than 25% of the older inoculated trees the cankers progressed below the soil line to the underground parts of the main roots. There was no significant difference in the rate of growth of the canker in the coffee varieties tested. However, it appears that in the variety "Arabica" the cankers grow at a slower rate.

Active perithecia of Ceratocystis were found in the natural habitat only in the rainy season. Perithecia were found abundantly in old diseased trees which had been pulled out and had recent cuts in the bark. Perithecia were found in June in the cankers of inoculated trees. They were more prevalent in cankers near the ground than on cankers on the upper parts of the stem and branches. They were generally absent in the upper stem and branch inoculations of sun coffee (grown without shade) presumably because of a less humid condition than when grown under shade. By September 9 fructional perithecia were generally absent on all cankers in inoculated trees, and none were found by March 25, 1960. These dates fall within periods which are normally dry. It would appear that the succulence of the plant part and especially the relative humidity at the surface of the canker is of particular importance in perithecial formation and ascospore production.

---

[1] This study was made possible through the cooperation of the Finca ONA, Cuatepeque, Guatemala, and the Asociacion Experimental Cafetalera de Guatemala.

[2] Technical Director of the Asociacion Experimental Cafetalera de Guatemala.

Literature Cited

1. ECHANDI, E. 1955. Trunk and stem canker of coffee trees. Rev. Biol. Trop. 3 (2): 237-241.
2. HEISER, D. G. 1945. A Nectria disease of coffee in western Guatemala. Ann. Missouri Bot. Garden 32: 287-296.
3. LeBEAU, F. J., M. A. FLORES, y M. R. HERNANDEZ. Junio de 1955. El cancer del cafe causado por Nectria. Ministerio de Agricultura, SCIDA Instituto Agropecuario Nacional Agric. No. 2. La Aurora, Guatemala.
4. PONTIS, R. E. 1951. A canker disease of the coffee tree in Colombia and Venezuela. Phytopathology 41: 178-184.
5. SZKOLNIK, MICHAEL. 1951. Coffee trunk and stem canker in Guatemala. Plant Disease Reptr. 35: 500-501.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY

RECENT OCCURRENCE OF BACTERIAL BLIGHT OF POINSETTIA IN FLORIDA[1]

Lorne A. McFadden[2] and Don B. Creager[3]

INTRODUCTION

The poinsettia, Euphorbia pulcherrima, is one of Florida's most attractive and popular ornamental plants. In the spring of 1958 our attention was drawn to a serious disease of poinsettia, heretofore not recognized as occurring in the State. Isolations made from infected stems in the laboratory consistently yielded a bacterium which proved pathogenic to poinsettia stems when artificially inoculated.

The nature of the disease is such that plants may be infected yet show no visible symptoms. When diseased stock plants are cut back and maintained in the nursery, new growth may appear normal for 1 to 12 months prior to disease expression. Frequently, only one branch of a plant becomes blighted and the remaining shoots appear normal for several months.

Once the pathogen is introduced into a nursery, the disease may cause extensive damage. During the past year the malady has curtailed the expansion of the poinsettia industry in the State by interrupting the production of healthy poinsettia cuttings and pot plants. Certification for movement is refused on such materials when propagated from infected stock plants, even though the cuttings or young plants may appear healthy.

DISTRIBUTION

A review of the literature revealed that a similar disease had been described previously from Maryland, New Jersey, Pennsylvania and New York by Pirone and Bender in 1941 (6) and Starr and Pirone in 1942 (7). More recently the disease was reported from Alabama (1), where it caused a damping-off of unrooted poinsettia cuttings under mist propagation, and from New Zealand (2).

Thus far, the disease has been located in 11 different home and nursery plantings in six different counties in Florida (Fig. 1). In all cases the disease was verified by isolation of the causal agent in the laboratory, and in most cases by inoculations in the greenhouse.

Since the disease has been found in such widely separated localities, in old as well as young plants, in home gardens as well as in nursery plantings, there is no indication at present that the disease in Florida has had its origin from any one source. There is no proof as to how or when the pathogen might have been introduced into the State. Photographs taken in 1941 by Dr. G. D. Ruehle, showing specimens with symptoms of an unknown poinsettia disease collected from the Spalding Estate in Miami, resemble in many respects the diseased plants described here. In all probability bacterial blight has been present in Florida for some years.

DISEASE SYMPTOMS

The disease causes a malformation and blighting of the growing point of one or more stems of a plant. The growing point curves downward and inward toward the affected area of the stem. The terminal leaves also are curved and develop characteristic light spots which later become necrotic and form brown blotches (Fig. 2). The affected leaves eventually drop, leaving the stem bare. A close examination of the green stem shows characteristic longitudinal, narrow, water-soaked streaks. The disease organism generally is confined within the stem streaks prior to blighting the terminal growing point; but at times large, irregular, water-soaked, brownish lesions form along the stem. Affected plants often show latex droplets which dry to a golden brown. Bacteria abound in these droplets. When infected stems are cut open, a pale tan discoloration is visible in the areas of the sub-epidermal, water-soaked streaks.

In general, the disease does not kill the plant, although several branches may be completely blighted. Infected stock plants are often of poor vigor and fail to make new breaks.

SPREAD OF THE DISEASE

In the nursery the pathogen is believed to spread to adjacent plants primarily on contaminat-

[1]Florida Agricultural Experiment Station Journal Series No. 1081.
[2]Assistant Horticulturist, Sub-Tropical Experiment Station, Homestead, Florida.
[3]Chief Plant Pathologist, State Plant Board of Florida, Gainesville, Florida.

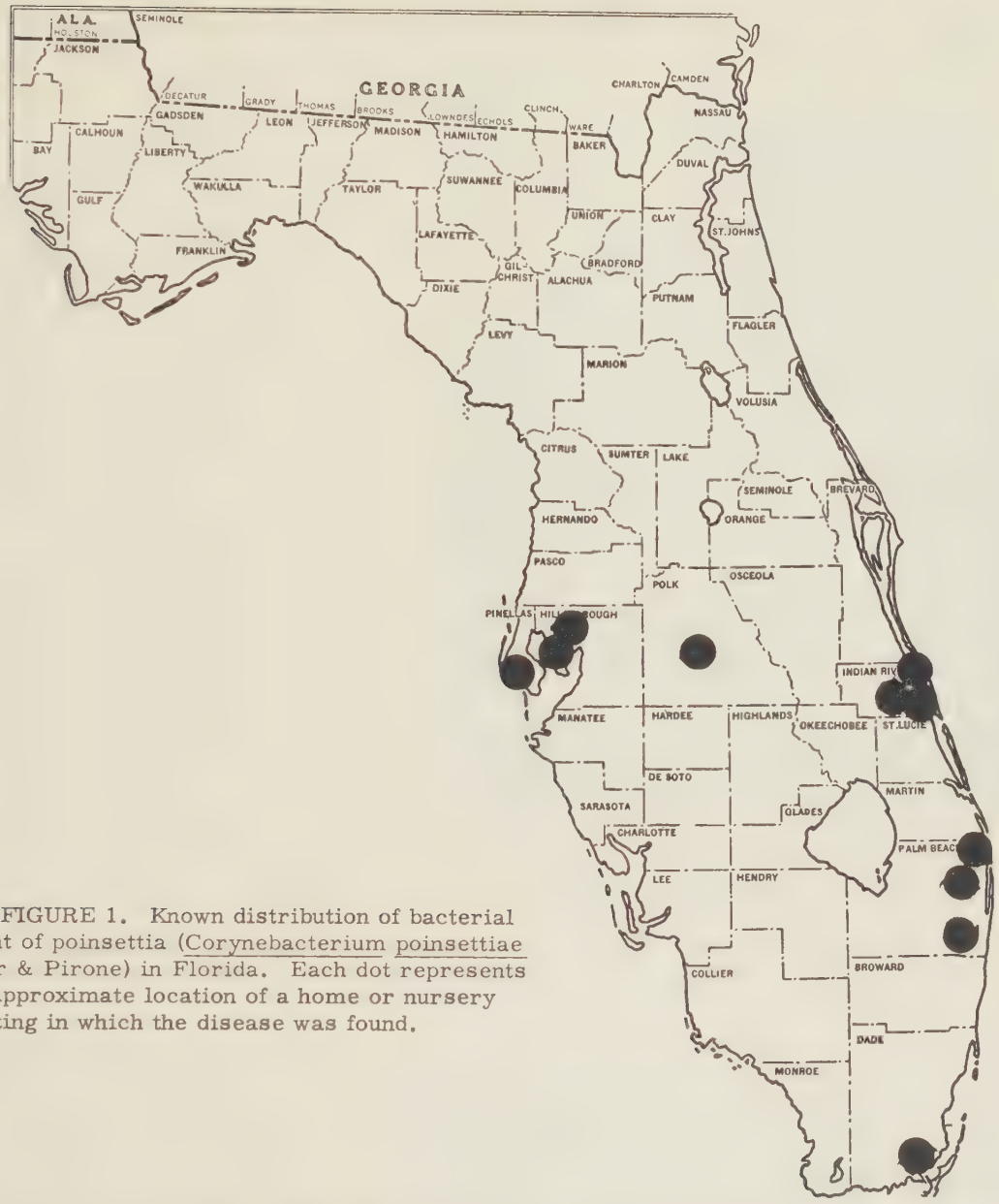


FIGURE 1. Known distribution of bacterial blight of poinsettia (Corynebacterium poinsettiae Starr & Pirone) in Florida. Each dot represents the approximate location of a home or nursery planting in which the disease was found.

ed knives or tools used when plants are cut back, or when tip cuttings are taken for propagation. In addition, disease spread may occur in the rooting bench if infected cuttings are interspersed with healthy cuttings. In a similar manner, the practice of nurserymen to "bleed" stem cuttings of latex by placing them in a pail of water also may serve to spread the organism. Cuttings inoculated with the pathogen were observed to root in many instances and to appear normal for several months before developing typical disease symptoms. Thus, infected cuttings may serve to spread the disease from one location to another.

Latex droplets containing the pathogen, which frequently occur on infected stems and leaves, could also serve as a source of inoculum. These droplets containing bacteria may be spread to adjacent leaves and plants by rain or irrigation water.

INOCULATION EXPERIMENTS

Attempts were made to infect healthy poinsettia stems by introducing the pathogen into wounded stems and leaves and "unwounded" leaves (3, 4). Visible infections occurred around

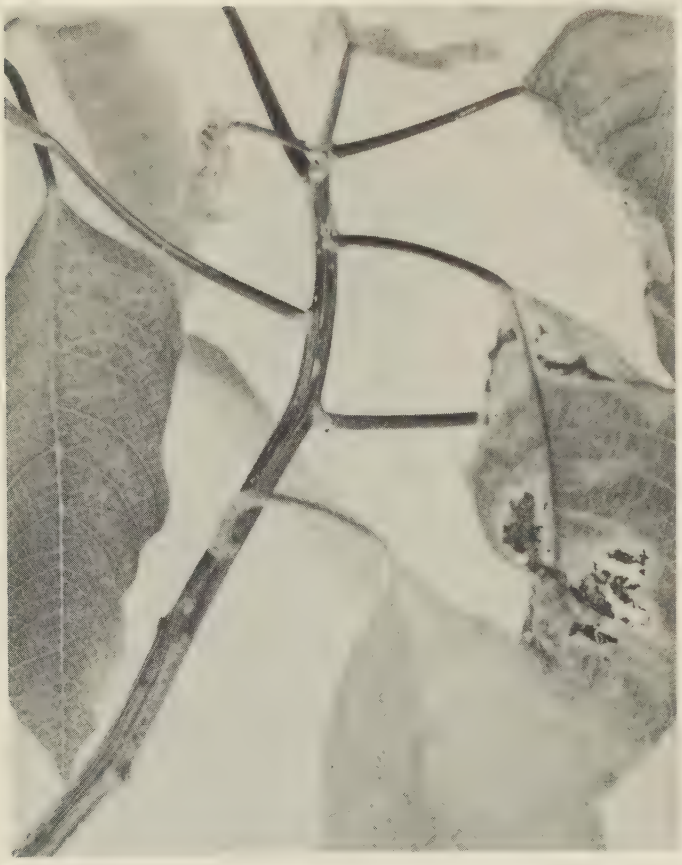


FIGURE 2. Poinsettia stem showing typical symptoms of bacterial blight. Note stem streaks, leaf blotches, and blighting and distortion of terminal growing point.

inoculated knife wounds in stems within 1 week, but blighting of the terminal growing point did not occur until 2 to 8 weeks later. Both wounded and unwounded leaves of plants sprayed with a water suspension of the pathogen developed characteristic brown blotches after 2 to 4 weeks. When succulent tip cuttings were inoculated by placing the freshly cut ends in a water suspension of the pathogen, typical water-soaked longitudinal lesions appeared within 3 weeks after placing the stems in a rooting medium under intermittent mist. Similarly, when succulent tip cuttings were removed with a knife contaminated with the bacterial pathogen, characteristic water-soaked streaks developed on the stock plants within 2 weeks. Typical brown blotches developed on leaves of infected stems and new bud breaks were delayed or prevented, depending on the severity of infection. The pathogen has been readily re-isolated from both stem and leaf lesions. In no instance was the disease observed to enter the stem from leaf lesions. In most instances infected leaves turned yellow and dropped prematurely. When added to the soil around plants, the pathogen failed to produce the disease after a 2-month period.

CONDITIONS FAVORING THE DISEASE

Under southern Florida conditions, this bacterial disease has been observed at various times of the year. However, it is more commonly found in the late spring, summer and early fall months. During these periods poinsettia propagators force stock plants by frequent applications of fertilizers in order to produce an abundance of soft, succulent tip cuttings. Such plants appear to be more susceptible to attack than those which are growing less vigorously. These observations were noted also by Pirone and Bender (6). High temperatures appear favorable for disease development, although it is not uncommon to see flowering plants showing the blight symptoms at a time of year when temperatures are considered moderate. Disease development is favored by but not dependent upon high humidity.

THE PATHOGEN

In 1942 Starr and Pirone (7) described a newly recognized bacterial pathogen isolated from diseased poinsettia plants and named the organism Corynebacterium poinsettiae n. sp. In an attempt to identify the pathogen inciting a similar disease of poinsettia in Florida, six isolates of equal pathogenicity were selected for a study involving the cultural, physiological and morphological characteristics of the blight organism. The results of this study showed that the pathogen causing bacterial blight of poinsettia in Florida is similar, with few exceptions, to Corynebacterium poinsettiae Starr & Pirone. Starr and Pirone's original description of this organism lists negative fermentation tests for mannitol, sorbitol, and inositol, whereas the Florida isolates gave positive tests for the fermentation of these compounds. In addition, the Florida isolates gave a negative test for starch hydrolysis, while Starr and Pirone found the test for starch hydrolysis to be positive.

Cultures of the Florida pathogen forwarded by the junior author to M. P. Starr in California were verified as Corynebacterium poinsettiae.

CONTROL

Owing to the systemic nature of the disease, and the fact that the organism remains in stock plants several months before expressing symptoms, commercial nurserymen and home owners are advised to remove and discard infected plants as they appear. Efforts should be made to obtain disease-free stock plants for future propagation.

Results of preliminary tests have shown that the disease was greatly reduced or inhibited by weekly foliage sprays (4). Two hundred and forty poinsettia stock plants, many of which were known to harbor the disease, were randomly set into 40 plots of 6 plants each. Ten spray treatments, each replicated four times, were applied weekly for a period of 6 months. The plants were cut back once during this test period. Data taken on the number of stems blighted at the end of this period showed that no disease occurred in plants sprayed with Agri-mycin 500 (10% streptomycin sulfate, 1% oxytetracycline, + basic copper sulfate (1% as metallic copper)) (4 pounds/100 gallons). Relatively few instances of disease occurred in plots sprayed with Agri-mycin 500 at 2 and 3 pounds per 100 gallons, Agri-Strep (streptomycin sulfate) (60 ppm active ingredient), tri-basic copper sulfate (4 pounds/100 gallons) and Agri-mycin 100 (15% streptomycin sulfate, 1.5% oxytetracycline) (60 ppm active ingredient). Natriphene (Sodium salt of o-hydroxydiphenyl) (1/2 pound/100 gallons), Elcide 70 (Sodium ethyl mercury thiosalicilate, 12%) (150 ppm), and Nabac (2' methylenebis (3,4,6 trichlorophenyl) 25%) (1/2 pound/100 gallons) were no better than the water-sprayed controls. Elcide 70 proved phytotoxic at the concentration employed. The remaining materials appeared safe over the entire test period. The reduction of blight in plots sprayed with Agri-mycin 500, Agri-mycin 100, and Agri-Strep is believed due to stem absorption of streptomycin contained in the formulations. This is further substantiated by tests using a 1% Agri-Strep-lanolin paste mixture similar to that described by Mitchell et al. (5). When this mixture was smeared on stems of poinsettia above the point of inoculation the disease was suppressed, whereas the untreated plants became blighted.

Attempts were also made to control the disease in cuttings in the rooting medium. When the basal ends of inoculated cuttings were dusted with rooting hormone powder supplemented with Agri-Strep at 1.0 and 2.0% active concentrations, disease control was slightly superior to that with cuttings receiving similar treatments of Agri-mycin 100 or 500 and Chloromycetin. None of the treatments were highly effective, however.

The poinsettia bacterium was completely inhibited within a 1-minute exposure period when Agri-Strep at 25 ppm active ingredient was added to water contaminated with the pathogen. Such materials added to the water in which cuttings are placed to "bleed" could conceivably reduce the spread of this pathogen.

Literature Cited

1. ANONYMOUS. 1959. November suggestions, Southern retail grower. Florists' Review 125 (3232): 27-28.
2. BRIEN, R. M., and JOAN M. DINGLEY. 1957. Third supplement to "A revised list of plant diseases recorded in New Zealand", 1955-1957. New Zealand J. Sci. and Tech., Sect. A., 38(7): 777-781.
3. CREAGER, DON B. 1959. Inoculation studies with Corynebacterium poinsettiae. Proc. Florida State Hort. Soc. 72: (In press).
4. McFADDEN, LORNE A. 1959. Bacterial blight of poinsettia. Proc. Florida State Hort. Soc. 72: (In press).
5. MITCHELL, J. W., W. J. ZAUMEYER, and W. P. ANDERSON. 1952. Translocation of streptomycin in bean plants and its effect on bacterial blights. Science 115: 114-115.
6. PIRONE, P. P., and T. R. BENDER. 1941. A new bacterial disease of poinsettia. Nursery Disease Notes (New Jersey Agr. Exp. Sta.) 14: 13-16.
7. STARR, M. P., and P. P. PIRONE. 1942. Phytomonas poinsettiae n. sp., the cause of bacterial disease of poinsettia. Phytopathology. 32: 1076-1081.

SUB-TROPICAL EXPERIMENT STATION, HOMESTEAD, FLORIDA AND STATE PLANT BOARD OF FLORIDA, GAINESVILLE

THE PRODUCTION AND USE OF ZOOSPORE SUSPENSIONS OF PHYTOPHTHORA SPP. FOR INVESTIGATIONS ON DISEASES OF CITRUS

L. J. Klotz and T. A. DeWolfe[1]

Investigations with Phytophthora spp. pathogenic to citrus frequently require large quantities of concentrated zoospore suspensions as inocula. Naturally occurring infections of all parts of citrus are most frequently caused by zoospores and occasionally by direct germination of zoosporangia. To secure zoospore suspensions, the following procedure is followed: The fungi are first grown on alfalfa stems to produce abundant mycelium. Stems from which the leaves have been removed are cut to 3-inch lengths and in packets of 4 to 6, depending on their girth, placed in test tubes containing approximately a half gram of calcium carbonate (for favorable pH) and ion-exchange water to a depth of 2 inches, and autoclaved. Bits of mycelium from agar, apple fruit, or other culture media are placed on the stems at the water surface. When incubated at 26° C for 4 or 5 days, P. citrophthora and P. parasitica produce sufficient mycelium. The alfalfa sticks are then dumped onto a piece of hardware cloth placed over the open top of a battery jar or other suitable container filled with water, and a small stream of water is allowed to fall from a height of 1 to 2 feet so as to aerate the water and culture. Within 4 or 5 hours, with the water temperature at 20° to 25° C, P. citrophthora begins to form zoosporangia. After 18 to 24 hours of the aerated washing, the sticks and fungus are removed to a moist chamber where, with the temperature at 22° to 25° for P. citrophthora or 25° to 27° for P. parasitica, the fungi produce sporangia in profusion during the next 48 hours. After 48 hours in the moist chamber, bacteria begin to cause malodorous decomposition of the alfalfa. The sporangia-laden mycelium of P. citrophthora on the alfalfa stems is next dumped into aerated water at 16° to 18° to induce formation and discharge of zoospores. Lowering the temperature of the aerated water to 23° or 24° is sufficient to cause swarming of P. parasitica. The highly concentrated spore suspension is then diluted with the cool water (16° to 18° for P. citrophthora; not below 20° for P. parasitica) to the concentration desired. Cool water prolongs the period of motility.

Many fungicidal spray and dust formulations have been tested for their effectiveness in protecting citrus fruit against brown rot infection induced with the concentrated zoospore suspensions. The principal procedure employed has been first to spray the fruit and foliage thoroughly with chemicals. After allowing for periods of weathering, which may or may not include rainfall, samples of sprayed and unsprayed fruit are brought to the laboratory and atomized[2] with a zoospore suspension, placed in a moist chamber, and left undisturbed for 48 hours at room temperature (20° to 26° C). Then on each of 3 successive days the infections on each fruit are counted and each infection marked with a dot of waterproof ink, thus requiring the addition to the total of only the newly appearing infections of the second and third counts. After another week a final inspection is made and any tardily developing infections marked and recorded.

In another method, the fruit is brought to the laboratory, sprayed with the fungicidal chemicals, and allowed to dry. It is next weathered under various amounts of artificial rain, atomized with a zoospore suspension, and finally the infections marked and counted as described. Efficacy of a spray treatment is based on the number of infections on sprayed fruit in relation to the number on fruit not protected by a fungicide. The mean number of infections on each untreated fruit of a sample is either reduced or elevated to 100 as the result requires and all counts on the treated fruit adjusted to this number in the final record. For example, if the unprotected (check) fruits have an average of 50 infections each and a treated lot 25 each the former would be rated 100 and the latter 50. If untreated fruits had a mean of 200 infections each and those of a treated lot 40 each, the check would still be rated 100 and the treatment 20.

The artificially produced zoospore suspensions are also used in estimating the resistance or susceptibility of the fibrous roots of various rootstocks to Phytophthora root rot. In one method, seedlings are fastened to paraffined blocks of wood or corks with a rubber band and floated on aerated water so that only the roots are immersed (2). Zoospores are added to the water and after a week the percentage of decayed roots estimated. A modification in general use is to dip or expose for several days the root systems of the varieties under test in a zoospore suspension and plant them in soil along with a similarly inoculated and very susceptible

---

[1] Professor and Associate Specialist, respectively, University of California Citrus Experiment Station, Riverside, California.

[2] The size of the droplets and air pressure at which they are applied to the fruit should be such that the zoospores are not injured. This may be determined by microscopic examination of droplets removed from the fruit.

sweet orange seedling for comparison. After 4 to 6 weeks in pots of soil, the root systems are washed out and percentage of rotted roots estimated. Or, after inoculation, the seedlings may be planted in a peat moss-vermiculite mixture, incubated for 2 months, and then an estimation of resistance may be based on percentage of survivors[3].

In laboratory studies, such as chemotaxis of zoospores and chemotropism of germ tubes (3), mode of infection, and rate of penetration, where relatively small quantities of uncontaminated zoospore suspensions may be required, the zoosporangia can be developed from mycelium on sterilized hemp seed in a thin layer of sterile water (8 to 10 ml for good aeration) in a standard 90 mm Petri dish. A modification of this method employs horse manure sterilized with propylene oxide (1) and used in place of hemp seed. Discharge of zoospores of P. citrophthora is effected by replacing the culture water with fresh water at 16° to 18° C. Zoosporangia of P. parasitica can be stimulated to discharge simply by changing the culture water with fresh water at the same temperature. In all operations with Phytophthora spp., copper-free water, preferably deionized water, should be used.

Literature Cited

1. HANSEN, H. N., and W. C. SNYDER. 1947. Gaseous sterilization of biological materials for use as culture media. Phytopathology 37: 369-371.
2. KLOTZ, L. J., T. A. DeWOLFE, and PO-PING WONG. 1958. Decay of fibrous roots of citrus. Phytopathology 48: 616-622.
3. ZENTMYER, G. A. 1960. Root exudates in relation to infection by Phytophthora cinnamomi. Phytopathology 50: (In press).

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION, RIVERSIDE

[3] Modification developed and used by Dr. John B. Carpenter, United States Department of Agriculture Date Field Station, Indio, California.

A COMPARISON OF POLYETHYLENE PLASTIC BAGS AND GLASS JARS AS INCUBATION CHAMBERS FOR OBTAINING NEMATODES FROM ROOTS[1]

A. C. Tarjan

Summary

A higher number of burrowing nematodes was obtained consistently from roots incubated in one mil-thick polyethylene plastic bags than from roots incubated in glass jars for periods up to 9 days at 75° F. Incubation of roots in plastic likewise proved superior to immersion in Baermann funnels. A higher number of nematodes was obtained when roots alone were incubated in plastic or glass jars than when roots were mixed with either infested subsoil or autoclaved soil. The advantages in the use of plastic bags in comparison with glass jars as incubation or collecting chambers are enumerated.

A widely used method for facilitating the exodus of nematodes from roots has been that originated by Young (3). In Florida, this technique of incubating the roots in sealed glass jars is employed by State and Federal personnel conducting research on the burrowing nematode, Radopholus similis (Cobb) Thorne. A recent account by the author (1) has shown the utility of polyethylene plastic bags for incubating combined soil and root samples as a means of improving the yield of burrowing nematodes. It was further shown in this paper that maximal yields of nematodes occurred after 6 to 7 days at a temperature of 75° F. The principal purpose of the present study was to compare one mil-thick polyethylene plastic bags, of approximately 2-quart capacity, with the standard pint glass Mason jars in order to determine which gave the higher yield of nematodes from a given quantity of roots.

PROCEDURES

Burrowing nematode-infected citrus feeder roots were obtained mostly from a 3-foot depth. These were shredded to 1/2-inch lengths and thoroughly mixed, to provide a root mass of relatively uniform nematode infestation. For each of the two experiments performed there were 10 replications of each treatment.

Test 1: Seventy grams of roots were placed either directly in glass Mason jars or plastic bags, or were mixed with previously autoclaved soil, to comprise a volume of 1 pint, and then placed in plastic bags. One pint of nematode-infested subsoil, free of roots, was also placed in plastic bags. The bags and jars were sealed and incubated at 75° F. After, 3, 5, and 7 days of incubation, the samples comprising each treatment were processed for nematodes. Samples consisting only of roots were washed three times with water, that was then passed once through a 60-mesh sieve and twice through a 325-mesh sieve. Nematodes retained on the latter sieve were collected and immersed in 100 cc of water. Nematodes present in soil were extracted by use of the elutriation device designed by Tarjan, Simanton, and Russell (2). Debris deposited on the collecting sieves of this elutriator was placed in Baermann funnels. The following morning, the liquid contents of each funnel were passed through No. 41 Whatman filter paper mounted in Büchner funnels. Where a mixture of roots and soil was incubated, roots which were separated from the soil were placed in a Baermann funnel for 3 days, after which the liquid contents of each funnel were passed through filter paper and the nematodes collected as described. Burrowing nematodes in two representative aliquots were counted and these counts were projected to estimate the total number of nematodes.

Test 2: Although essentially modeled after the first test, only 12.5 g samples of nematode-infested roots were utilized for each replicate in this experiment. These were placed either in plastic bags, glass Mason jars, or mixed with enough nematode-infested subsoil to comprise 1 pint of mixture and then placed in plastic bags. As in the previous test, 1 pint of nematode-infested subsoil, free of roots, was also placed in plastic bags. The bags and jars were sealed and then incubated at 75° F. After 6 days the roots in 10 replicate plastic bags were washed and the nematodes accumulated and counted as described in the previous test. These roots

---

[1] Florida Agricultural Experiment Stations Journal Series, No. 1076.

were resealed in the same bags, incubated for an additional 3 days, and again processed for nematodes. The roots in 10 additional plastic bags and 10 glass jars were similarly treated, except they were placed, after washing, in Baermann funnels for the additional 3-day incubation. The infested soil and roots in plastic bags were separated, the soil processed for nematodes in the elutriation device, and the roots placed in Baermann funnels for 3 additional days. The remaining treatment consisting of root-free, infested subsoil was processed for nematodes in the elutriation device. In all but the latter treatment counts of burrowing nematodes obtained after the 6- and 3-day incubation periods were combined so as to represent the total count for 9 days.

RESULTS

Test 1: Perhaps the most striking data from this test were the higher average number of burrowing nematodes per replicate obtained from roots incubated in polyethylene plastic bags as compared with roots incubated in glass jars. Table 1 shows this difference to be significant at odds of 19 to 1 (LSD .05) when roots were harvested after 3 days' incubation, and significant at odds of 99 to 1 (LSD .01) when harvested after 5 and 7 days' incubation. When roots were incubated in previously autoclaved soil, presumably devoid of most microorganisms, the average number of nematodes obtained from both the roots and soil was significantly lower, at odds of 99 to 1, than the numbers obtained from the same amount of roots incubated in plastic or in glass jars. Burrowing nematodes were also present in the root-free subsoil comprising the rhizosphere although in much reduced numbers as compared with the nematode counts from the roots (Table 2).

Table 1. Average counts of Radopholus similis from roots and/or soil incubated in plastic bags or glass jars for various periods of time (Test 1).

Incubation period (in days)	Treatment[a]		
	Roots in plastic	Roots in jars	Autoclaved soil and roots in plastic
3	925	690	231
5	1030	550	200
7	1230	805	338

LSD.05 = 207
LSD.01 = 274

[a]Mean of 10 replicates.

Table 2. Average counts of Radopholus similis from root-free subsoil incubated in plastic bags (Test 1).

Incubation period (in days)	Nematode count[a]
3	58 ± 7
5	84 ± 13
7	76 ± 15

[a]Mean of 10 replicates ± standard error.

Table 3. Average counts of Radopholus similis from roots and/or soil incubated in plastic bags or glass jars for a total of 9 days (Test 2).

Incubation period	Treatment[a]				LSD .05	LSD .01
	Roots in plastic	Roots in plastic	Roots in jars	Infested soil and roots in plastic		
Initial 6 days	603	626	485	373	169	229
Additional 3 days	86[b]	30[c]	38[c]	19[c]	29	39
Totals for 9 days	689	656	523	392	204	276

[a]Mean of 10 replicates.
[b]Incubated in plastic.
[c]Incubated in Baermann funnels.

Test 2: When 6 days were utilized for incubation of samples, nematode counts from roots incubated in plastic were again appreciably higher than those incubated in jars. Statistical significance was not evident, apparently due to the variability in counts from individual replicates per treatment (Table 3). The superiority of an additional 3-day incubation of roots in plastic as compared with immersion in Baermann funnels was highly significant. The total counts of nematodes obtained after 9 days' incubation failed to show statistical significance when plastic bag treatment was compared with the glass jar treatment although, as before, nematode counts for the former were appreciably higher than for the latter. When roots were incubated in infested soil, resulting nematode counts were lower at the 1% level of significance than those obtained from the roots incubated in plastic bags. Incubation of root-free, infested soil for 6 days yielded an average of 81 ± 13 burrowing nematodes per replicate.

DISCUSSION OF RESULTS

Test 1: Since it had been previously found that maximum nematode yield from root and soil samples occurred after 6 to 7 days (1), Test 1 was designed to compare the nematode yield from roots incubated in plastic bags with those incubated in glass jars at various periods of time, up to a maximum of 7 days. This test showed conclusively that plastic bag incubation enhanced burrowing nematode yield as compared with glass jar incubation. Although plastic bag incubation of roots and soil has increased the number of nematodes extracted in previous tests (1), in the present experiment this number was significantly lower in comparison with the number of nematodes obtained from an equivalent amount of roots incubated without accompanying soil. Accordingly, Table 1 shows that incubation of roots with autoclaved soil (free of burrowing nematodes) can reduce nematode yield 58 to 66%, compared with yields from roots incubated in glass jars, and 73 to 81% compared with yields from plastic bags. Even if the roots had been incubated with the infested subsoil, with which they were associated prior to digging, the values referred to above would not have changed appreciably. This is evident from the low count of burrowing nematodes obtained from incubated subsoil (Table 2).

Test 2: The purpose of Test 2 was to confirm the findings in Test 1 that a 7-day period of incubation gave the highest nematode yield from samples. A common procedure for processing root and soil samples in the author's laboratory has been to subject the roots to an additional 3 days of incubation in Baermann funnels following separation from the soil. Test 2 compared the efficiency of the Baermann funnel with that of an additional incubation in plastic bags for obtaining the maximum number of nematodes. In view of the low yield of nematodes obtained from roots mixed with autoclaved soil in the previous test, in Test 2 roots were mixed with nematode-infested subsoil, in order to determine whether this would enhance the yield. The results of Test 2 were comparable to those of the previous test in that more nematodes evacuated roots in plastic bags than in glass jars, although the differences were not statistically significant, presumably due to the variability among replicates (Table 3). Perhaps the fact that only 12.5 g of roots were used per replicate in this test as compared with the 70 g used in Test 1 may have been partly responsible. Yet, after the initial 6 days' incubation, when roots were incubated an additional 3 days, the number of nematodes evacuating the plastic bag incubation treatment was found to be significantly higher at odds of 99 to 1 than the other treatments, which involved immersion of roots in Baermann funnels. When counts from the 6-day and additional 3-day incubations were totaled to represent a 9-day incubation period, the same relationships between nematode yields from plastic bags and glass jars existed as existed after 6 days' incu-

bation; however, there was no evidence of statistical significance. As in the first test, incubation of roots mixed with soil significantly decreased the number of nematodes evacuating the samples as compared with nematodes evacuating the roots encased in plastic. However, the relatively greater number of nematodes from roots (12.5 g) and soil in Test 2 (Table 3) as compared with Test 1 (Table 1), in which 70 g of roots were employed, suggested that use of infested subsoil rather than autoclaved soil had resulted in a greater exodus of nematodes from roots, even when the 81 ± 13 burrowing nematodes present in the subsoil in Test 2 was taken into account.

The research reported in this paper demonstrates that use of one mil-thick polyethylene plastic "pre-packaging" bags in preference to glass jars will result in the extraction of more burrowing nematodes from a given sample. As pointed out previously (1), this may be due to the ability of plastic to delay dissipation of soil gases and yet admit oxygen, in contrast to glass which is impervious to gases. Despite the fact that any method allowing for a more accurate estimation of nematodes within a root sample should be the primary point for consideration, the feasibility of converting extraction procedures from glass jars to plastic bags would naturally be questioned. Plastic bags are considerably cheaper than glass jars, are not subject to breakage, do not require as much storage room as jars, can conveniently be labeled with a wax pencil, and can even be used several times if reasonable care is exercised to prevent tearing. Any root processing procedure normally carried on using glass jars likewise can be as effectively and quickly performed with the roots in plastic bags.

Literature Cited

1. TARJAN, A. C. 1960. Incubation of soil and root samples in polyethylene plastic for improved recovery of nematodes. Plant Disease Reptr. 44: 31-35.
2. TARJAN, A. C., W. A. SIMANTON, and E. E. RUSSELL. 1956. A labor-saving device for the collection of nematodes. Phytopathology 46: 641-644.
3. YOUNG, T. W. 1954. An incubation method for collecting migratory endo-parasitic nematodes. Plant Disease Reptr. 38: 794-795.

FLORIDA CITRUS EXPERIMENT STATION, UNIVERSITY OF FLORIDA, LAKE ALFRED

WINTER OUTBREAKS OF CITRUS BROWN ROT IN FLORIDA[1]

Mortimer Cohen and L. C. Knorr[2]

Abstract

Brown rot of citrus fruits on the tree was first encountered in Florida in 1954; since then the disease has been noticed annually. All outbreaks occurred characteristically in late summer and were caused by Phytophthora parasitica Dast. In 1959 and again in 1960, however, outbreaks appeared in late winter. The fungus involved in the winter outbreaks failed to sporulate in the manner characteristic of P. parasitica; its identity remains to be determined.

INTRODUCTION

Brown rot of fruits on the tree, a disease long troublesome in other citrus-growing areas of the world, was not reported from Florida until 1954 (4). Its subsequent progress in the State was summarized in 1956 (3). These two reports described outbreaks that occurred in late summer or early autumn when temperatures were high and drizzly rains were frequent; they were caused by Phytophthora parasitica Dast., a "hot-weather" representative of the genus; and attacks were limited to early and mid-season varieties of citrus, including Hamlin and Pineapple sweet oranges, Temples, and grapefruits. Late season varieties such as Valencias and Lue Gim Gongs were not attacked, even when growing beside badly affected varieties. This "resistance" of late oranges appeared in sharp contrast to what was described from California, where brown rot is known to attack Valencias and often to cause serious losses. It remained a matter of speculation as to why Valencia oranges appeared resistant in one part of the country and susceptible in another.

Since 1958, two outbreaks of brown rot have been discovered during cool, wet weather in late winter. The late-winter attacks were caused by a species of Phytophthora that failed to sporulate in pure culture, even when held at the elevated temperatures at which P. parasitica sporulates. The attacks occurred on Valencia oranges and on grapefruit at a time when other varieties were already picked.

OBSERVATIONS

Florida's winters are usually dry and sunny, but in March of 1959 an exceptionally prolonged, record-breaking spell of rainy weather occurred. The Weather Bureau at Lakeland (in Central Florida) recorded only 11 hours without precipitation during the 117 hours between 5 p.m. March 15 and 2 p.m. March 20. Most citrus groves remained wet for from 3 to 5 days. Total precipitation was 7.9 inches. At Fort Pierce (on the East Coast) rains lasted, with little interruption, from 7 p.m. March 16 to the early morning of March 20; and rainfall totaled 5.4 inches. During these periods, temperatures ranged from 57° to 75° F at Fort Pierce and from 48° to 68° at Lakeland.

Brown rot appeared on Valencia oranges and grapefruits March 25. Symptoms were typical of those described for the disease (2) except for absence of sporulation on affected fruits in the tree. Leaves and young shoots, as well as fruits, were attacked. This late in the season, all early and mid-season oranges were already picked; symptoms, therefore, were found only on leaves and twigs of such varieties as Hamlin, Temple, and Shaddock.

Grapefruit leaves and shoots were not affected except in one location near Vero Beach where Marsh grapefruit trees had received overhead irrigation several days in advance of the rains. This attack suggests the possibility that "resistance" in grapefruit foliage is a function of time needed for penetration of these leaves by the fungus.

Crop losses in the Indian River area were not severe during the 1959 late winter outbreak; they averaged less than 1% within affected groves. In the abovementioned irrigated block near Vero Beach, however, some 1500 boxes were lost to brown rot, representing 20% of the total crop.

---

[1] Florida Agricultural Experiment Stations Journal Series, No. 1095.

[2] Respectively, Associate Plant Pathologist, Indian River Field Laboratory, Fort Pierce, and Plant Pathologist, Citrus Experiment Station, Lake Alfred, Florida.

Losses in the Ridge district of Central Florida were also light, but the two outbreaks that occurred (one at Kissimmee and the other at Lake Alfred) marked the first time that brown rot had been seen in the intensive production area of the Ridge. An outbreak in Pasco County (on the West Coast) was also reported, but here, too, damage to the crop was small.

In 1960 another prolonged wet spell occurred in March. Between the 15th and the 18th of the month rains fell almost continuously, and precipitation totaled 11.26 inches at Lakeland. Temperatures during this interval ranged from 61° to 72° F. The relative humidity between 6 a.m. March 15 and 10 a.m. March 18 was almost continuously 100%. On April 1 brown rot was found on fruit in trees at Bartow. Again, crop losses were small.

DISCUSSION

Outbreaks of brown rot of fruit on the tree are not common in Florida; when they do occur, they take place in summer more often than in winter. This summer periodicity may be correlated with the greater frequency of prolonged rains in summer than at other seasons of the year. Another factor may be the stage of maturity of fruits at the time when ecological factors are appropriate for infection. This is suggested by the fact that Valencia oranges in Florida are not attacked in autumn, as are early-maturing Hamlin oranges and Temples, but do become susceptible late in winter. In California, too, outbreaks of brown rot in Valencias occur typically in late winter.

The species of Phytophthora involved in winter outbreaks of brown rot may be different from that responsible for summer outbreaks. The fungus on brown-rot affected fruits collected in summer sporulated readily when held overnight in moist chambers at 85° F. Contrariwise, the fungus on fruits collected during winter outbreaks does not sporulate at 85° F nor at temperatures down to 40°; neither will pure cultures sporulate, though they occasionally produce sporangia when contaminated with bacteria. The identity of the winter strain remains to be determined. It is suspected to be a species with low-temperature requirements for sporulation -- as is the case, for example, with P. citrophthora (Smith & Smith) Leonian. The last-named species does in fact exist in Florida (1), but so far it has been recovered only from rotting fruits on the ground and from trunks of citrus trees affected by foot rot.

Literature Cited

1. FAWCETT, H. S. 1933. New locations for Phytophthora citrophthora and P. hibernalis on citrus. Phytopathology 23: 667-669.
2. FAWCETT, H. S. 1936. Citrus Diseases and Their Control. McGraw-Hill Book Co., New York. pp. 411-412.
3. KNORR, L. C. 1956. Progress of citrus brown rot in Florida, a disease of recent occurrence in the State. Plant Disease Reptr. 40: 772-774.
4. WEST, ERDMAN, MORTIMER COHEN, and L. C. KNORR. 1954. Brown rot of citrus fruit on the tree in Florida. Plant Disease Reptr. 38: 120-121.

INDIAN RIVER FIELD LABORATORY, FORT PIERCE, FLORIDA AND
CITRUS EXPERIMENT STATION, LAKE ALFRED

WOODY GALLS ON CITRUS ASSOCIATED WITH VEIN-ENATION VIRUS INFECTION

James M. Wallace and R. J. Drake[1]

Abstract

Woody galls in varying stages of development have been observed on field trees of Rough lemon and West Indian lime, some of which are known to be infected with the virus which causes vein enation on leaves of various citrus species and varieties. Development of similar galls on young trees of Rough lemon and Mexican lime experimentally infected with vein-enation virus indicates that the galls are caused by this virus.

INTRODUCTION

The citrus vein-enation virus was described in California in 1953 (5) and in the Union of South Africa in 1954 (4), where it was demonstrated to be transmitted by the vector of tristeza virus, Toxoptera citricidus (Kirk.). Later, Wallace and Drake (6) demonstrated that this virus is transmitted in California by the green peach aphid, Myzus persicae (Sulz.). In both countries it has been shown that vein-enation virus is rather commonly present in numerous varieties and species of Citrus. Other than the enations, which consist of small tooth-like projections on the leaf veins, no symptoms or injurious effects have been attributed to the causal virus. However, some recent studies in California indicate that the vein-enation virus causes the formation of tumor-like galls on certain kinds of citrus. Rough lemon and sour lime are particularly subject to gall development. This paper describes preliminary field observations and experimental studies on the association of vein-enation virus with the development of woody galls on citrus.

HISTORICAL REVIEW

Fawcett and Bitancourt (1) illustrated an abnormality found on sweet orange trees which they described as "knobby bark." The specimen illustrated by these authors resembles the early stages of the galls to be described in this paper. Later, Fawcett and Klotz (2) described "knobby bark" and mentioned that this condition had been observed on both orange and lemon. Fawcett (unpublished) attempted to transmit and reproduce this disorder on sweet orange but was not successful.

While visiting citrus-producing countries in 1959, the senior author of this paper was shown a specimen of galls on Rough lemon found in a citrus nursery in Peru. He was informed that a low percentage of navel orange trees propagated on Rough lemon had developed this kind of growth on the Rough lemon rootstock within 2 years after propagation. Later that year, at the Citrus Research Station, Nelspruit, South Africa, three Rough lemon seedlings with extensive development of the tumorous type of galls were examined. These trees were in a large planting of Rough lemon seedlings originating from numerous sources which were being studied by W. J. Basson, Horticulturist.

In 1958 Fraser (3), in Australia, briefly described a disease on Rough lemon which had been given the name "woody gall." This author stated that this had been shown to be due to a bud-transmissible virus and that its presence in Rough lemon seedlings suggests that it has an active insect vector. Other than the brief description cited, the results of study on this disease in New South Wales have not been published. It seems very likely that the woody gall mentioned by Fraser is the same symptom as that described herein and for that reason the name "woody gall" is used in this paper.

SYMPTOMATOLOGY

Vein Enation: The leaf symptoms of vein enation are illustrated in Figure 1. They consist of slight vein swelling and the formation of small projections from the veins on the under surface of the leaf. The enations may be few or many and the individual projections vary in

[1]Respectively, Plant Pathologist and Principal Laboratory Technician, University of California, Riverside, California.

FIGURE 1. Leaf symptoms of vein-enation disease on leaves of Mexican lime: Left -- Vein swellings and enations on under surface of leaf. Right -- Same leaf showing corresponding indentations on upper surface.

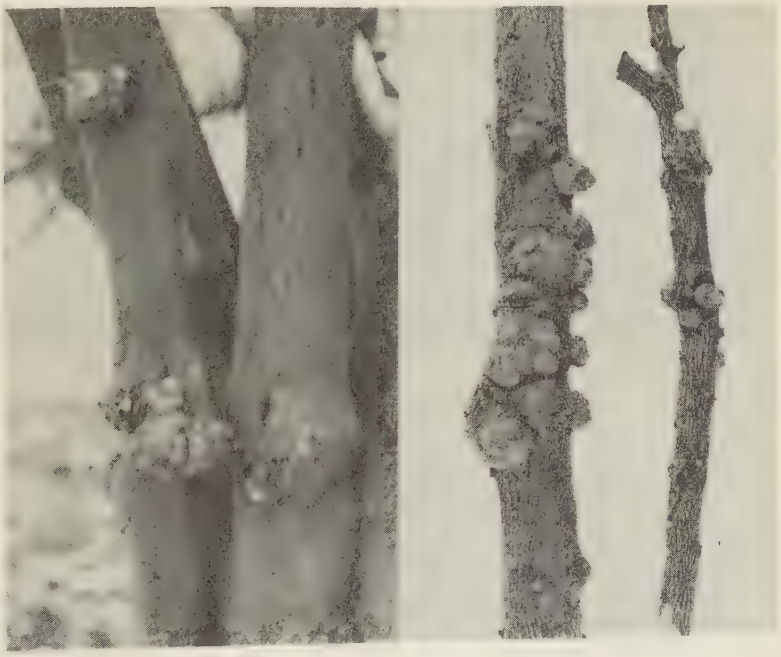


FIGURE 2. Naturally occurring woody galls on a field tree of sour lime at the Citrus Experiment Station, Riverside, California. This tree is 24 years old. It carries the vein-enation virus but there is no information as to when it became infected.

size, or length, from very slight to as much as 1 mm. Corresponding to the projections from the under surface of the leaf (Fig. 1, left) are indentations on the upper surface (Fig. 1, right). Numerous citrus species and varieties develop vein enations when infected but they vary in their reactions. Sour orange, Mexican lime, and Rough lemon are very good indicators, while symptoms are less conspicuous or rare on infected trees of sweet orange and sour lemon. In California the virus has been found in field trees of numerous kinds of citrus. In some districts there is a high percentage of infection, whereas in other localities it is rarely found. Fraser (3) states that the vein-enation virus is widespread in the citrus of New South Wales. McClean (4) found the virus rather commonly in South African citrus.

Woody Galls: The galls begin as slight swellings which gradually enlarge into knob-like projections covered for a period of time with smooth, unbroken bark. With additional enlargement, the knobby growths begin to roughen or develop unevenly and are often lighter in color than normal bark. Adjacent galls may coalesce and growth is irregular, producing, as described by Fraser (3), "cauliflower-like structures." Naturally occurring galls on a field tree of sour lime at Riverside, California are illustrated in Figure 2. Affected trees have not been

FIGURE 3. Naturally occurring and experimentally induced woody galls on young trees in California: Left -- Galls on rootstock of nursery tree of lemon on Mexican lime. Time of infection not known. Right -- Galls developing on a Rough lemon seedling graft-inoculated from a lime seedling that had been infected with vein-enation virus by means of the aphid vector, Myzus persicae. Tree on right photographed 21 months after inoculation.

under observation long enough to determine the ultimate effects on the trees. In South Africa the appearance of these galls on seedling trees which received no experimental inoculation suggested that the galls were caused by an insect-transmitted virus.

Examinations of old field trees of Rough lemon in California also revealed the "cauliflower" type of gall. On the same tree there were early-stage galls, which consist of single, small knobs of varying sizes and also the lighter-colored, irregular-shaped growths. The latter have been found in a wide range of sizes. The larger galls appear to have increased in size by a continual development of individual knobby growths from the gall tissues.

Other types of bark proliferations of unknown cause or origin are common on Rough lemon trees in California. Some are round or oval, varying from 2 to 20 mm in basal diameter and

extending outward from the bark in proportion to their size. Another type develops as a narrow, raised band, sometimes lenticular in shape and extending circumferentially on the trunk or limbs. Both of these seem to be different from the galls associated with vein-enation virus. They can be easily loosened by inserting a knife blade under them and applying a slight pressure. On the other hand, the galls associated with vein enation seem to be firmly attached and are not removed with such facility. No anatomical studies have as yet been made of any of the gall-like structures.

TRANSMISSION EXPERIMENTS

In tests made in California, seedlings of Rough lemon and Mexican lime have developed galls following inoculation with vein-enation virus. The sources of inoculum in many instances were old-clone field trees which may or may not have been carriers of other viruses in combination with vein-enation virus. Inoculations made by tissue grafts would have infected the test plants with any or all of the viruses present. However, tests which very probably excluded other viruses have given positive results and have provided good evidence that the galls are caused by the vein-enation virus.

Inoculations from a nucellar navel orange tree that had become naturally infected with the virus of vein enation resulted in gall development on Mexican lime and Rough lemon seedlings. This source of inoculum was shown to be free of tristeza and psorosis and, being of nucellar origin, the chances are good that it is not a carrier of other citrus viruses.

Inoculations from an exocortis-infected tree known to be free of vein-enation virus failed to produce either vein-enation symptoms or gall formations. Strains of tristeza virus, both mild and severe, have not caused development of galls.

The most conclusive evidence that the galls are caused by the vein-enation virus is that after this virus had passed through the aphid vector, Myzus persicae, to citrus seedlings, tissue-graft inoculations from these seedlings resulted in gall formation on lime and Rough lemon seedlings. Figure 3 shows the early stages of gall development on a young budded tree and on a small seedling tree of Rough lemon.

Inoculated plants vary in both the number of galls as well as in length of time required before the galls begin to develop. On some plants, the first stages of gall development have been detected within 2 months after inoculation. On others, a year or more has elapsed before the first gall developed. Earlier development of galls seems to be correlated with an increased number of galls.

DISCUSSION AND CONCLUSIONS

The woody galls on citrus which appear to be associated with vein-enation virus infection differ from other types of tissue proliferation found commonly on trees of Rough lemon and other citrus in surface shape and color, in the kind of connection between the woody tissue of the gall structure and normal tissues, and in rate of development and ultimate size.

Additional studies must be made before any conclusion can be made in regard to the practical importance of this disorder. At present Rough lemon is the only widely used commercial rootstock known to be subject to woody gall development. The fact that in addition to Rough lemon these galls have been produced experimentally in California on Mexican lime and have been found on several different kinds of citrus suggests that numerous citrus types may be subject to woody gall.

These studies seem to establish that this disorder is caused by a virus. Future experiments will be designed to substantiate the evidence that woody gall results from infection with the vein-enation virus. Host range and the effects of gall development on susceptible citrus will be determined.

Literature Cited

1. FAWCETT, H. S., and A. A. BITANCOURT. 1943. Comparative symptomatology of psorosis varieties on citrus in California. Phytopathology 33: 837-864.
2. FAWCETT, H. S., and L. J. KLOTZ. 1948. Diseases and their control. Chapter XI (pp. 495-596) In The Citrus Industry, Vol. II. Edited by L. D. Batchelor and H. J. Webber. University of California Press, Berkeley and Los Angeles.

3. FRASER, LILIAN. 1958. Virus diseases of citrus in Australia. Proc. Linnean Soc. N. S. Wales 73(part 1): 9-19.
4. McCLEAN, A. P. D. 1954. Citrus vein-enation virus. S. African J. Sci. 50: 147-151.
5. WALLACE, J. M., and R. J. DRAKE. 1953. A virus-induced vein enation in citrus. Citrus Leaves 33(2): 22, 24.
6. WALLACE, J. M., and R. J. DRAKE. 1959. Citrus vein enation. In Citrus Virus Diseases, pp. 163-165. Edited by J. M. Wallace. University of California Div. of Agr. Sciences.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION, RIVERSIDE

THE APOTHECIAL STAGE OF BOTRYTIS SQUAMOSA, CAUSE OF TIP AND LEAF BLIGHT OF ONIONS

D. M. McLean[1]

Summary

The perfect stage of Botrytis squamosa, the cause of tip and leaf blight of onions, was produced from sclerotia on 2% water agar at 72° F after 8 to 10 weeks. The apothecial stage is referred to as Sclerotinia squamosa (Viennot-Bourgin) Dennis. Apothecial characters alone do not differentiate this organism from Sclerotinia sclerotiorum emendavit Purdy; however, conidial and single ascospore isolates produced cultures and conidiophores typical of B. squamosa.

A tip and leaf blight of onions (Allium cepa) was unusually severe in 1958-59 in the Lower Rio Grande Valley of Texas (4). A species of Botrytis on affected leaves agreed in all respects with B. squamosa Walker which he reported (9, 10) to cause small-sclerotial neck rot of onion bulbs. This species is characterized by large conidia and septate conidiophores with curious accordian-like pleats or folds in degenerated side branches, a useful diagnostic character and the one that first gave a clue to the identity of this Botrytis. The fungus grows well on potato-dextrose agar (PDA) and produces numerous black sclerotia 1-5 mm in diameter.

B. squamosa was described by Walker (9) as producing numerous flat, scale-like sclerotia on infected onion bulbs. He tested its pathogenicity on onion foliage by spraying leaves with a spore suspension (10). After finding that leaves were not infected unless plants were held in a moist chamber at 18° C for a week or more, he remarked that "in an extremely humid environment having a favorable temperature, infection of the aerial parts by B. squamosa may occur," but that "there is little evidence that this form causes a destructive leaf blight." He presented no evidence of leaf infection by this fungus in the field during the growing season of onions.

Hickman and Ashworth (3), working in England, attributed disease symptoms similar to those observed on onion leaves in Texas to three species of Botrytis. The predominant fungus they isolated was B. squamosa. Viennot-Bourgin (7), who observed a firing of the leaf tips on white onions in the winter of 1951-52 near Rennes, France, considered B. squamosa the causal agent. Page (5) attributed a foliage disease of onions in the Holland-Bradford March, Ontario, during 1952 and 1953, to this fungus. In the reports cited, the investigators failed to find sclerotia on infected foilage in the field; typical "squamose" sclerotia were occasionally found on stored bulbs, however, and were produced readily in culture. In studies in Texas, numerous small, black sclerotia developed on diseased leaves and bulbs placed in moist chambers, and sclerotia were abundant on the necks of infected onions in the field.

Cronshey (1), in England, recorded the perfect stage of B. squamosa for the first time, when he observed apothecial development from sclerotia of two separate cultures of this fungus on Dox's agar after 7 to 10 weeks. Apothecial characters were consistent with those of Sclerotinia. Single-ascospore cultures developed sclerotia that produced conidiophores typical of B. squamosa. Leaf infection on onion seedlings was obtained by ascospore inoculation, and the fungus was reisolated from the lesions, which under humid conditions became covered with conidiophores.

In March 1959 sclerotia from laboratory cultures of B. squamosa were placed on 2% water agar in cotton-plugged screw-capped 4-ounce bottles. The bottles were placed in daylight at 40° F. On May 20 (8 weeks later) the sclerotia were examined and the bottles removed to the laboratory at 72° and placed in a lighted window for several weeks. Abundant Botrytis conidiophores and conidia developed from the sclerotia, but no apothecial primordia were observed. On May 27 other groups of sclerotia from the same cultures held in a refrigerator were placed on water agar in similarly stoppered bottles. These bottles were kept continuously in a lighted window in the laboratory at 72°. After 8 to 10 weeks, stipes 3-4 mm long were arising from a number of sclerotia. Several developed typical apothecial cups bearing asci and mature ascospores. The apothecia appeared to be consistent with those of Sclerotinia as reported by Cronshey (1). This is the first observation of the perfect stage in North America.

[1]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, in cooperation with the Texas Agricultural Experiment Station, Weslaco, Texas.

Sclerotial size (1.0-5.0 mm) falls within the collective measurements given for Sclerotinia minor Jagger (0.25-5.0 mm) or S. sativa Drayt. & Groves (1.0-5.0 x 1.0-2.0 mm) (6). Asci measured 162.5-200.0 μ x 13.8-16.5 μ and contained eight ascospores averaging 15.0-17.5 μ x 10.0-12.5 μ. These measurements fall within the broad range for similar structures in the species S. sclerotiorum (Lib.) d By., S. trifoliorum Eriks., S. trifoliorum var. fabae Keay, S. minor, S. intermedia Ramsey, and S. sativa, which because of the wide range of variability (asci 81.0-252.2 μ x 4.3-22.4 μ, ascospores 6.0-28.2 μ x 2.0-15.2 μ) were grouped under a single species as S. sclerotiorum (Lib.) d By. emendavit Purdy (6).

Whetzel in 1945 (11) erected the genus Botryotinia to segregate the fungi forming a Botrytis (cinerea type) asexual stage from the genus Sclerotinia Fuckel. Cronshey (1) apparently was not aware of Whetzel's reclassification of the species formerly included in Sclerotinia. Later, Viennot-Bourgin in France (8) described and illustrated with considerable detail the perfect stage of Botrytis squamosa as Botryotinia squamosa n. sp., but he did not cite the reference to Cronshey's (1) Sclerotinia.

Whetzel's (11) delimitation of the genus Botryotinia is not based on characters of the apothecia but on stromatal and asexual characters. In Botryotinia the medullary hyphae in the sclerotia are "embedded in a hyaline flexible to gelatinous matrix" and there are no interhyphal spaces, while the conidial stage is of the Botrytis (cinerea) type. In Sclerotinia sclerotia are of "densely interwoven hyphae with occasional small interhyphal spaces, hyphae not embedded in a gelatinous matrix" while a conidial stage is unknown. Certain mycologists would disagree with Whetzel's fundamentals for erecting the genus Botryotinia and place the fungus under discussion in the genus Sclerotinia. Dennis (2) records the onion fungus in question as Sclerotinia squamosa (Viennot-Bourgin) comb. nov. and refers to Botryotinia as a subgenus.

Specimens of Sclerotinia squamosa observed in Texas are deposited in the National Fungus Collections, Plant Industry Station, U.S.D.A., Beltsville, Maryland, and in the Herbarium of the University of Wisconsin, Madison.

Literature Cited

1. CRONSHEY, J.F.H. 1946. The perfect stage of Botrytis squamosa Walker. Nature 158: 379.
2. DENNIS, R.W.G. 1956. A revision of the British Helotiaceae in the herbarium of the Royal Botanic Gardens, Kew, with notes on related European species. Commonwealth Mycological Institute, Mycological Papers No. 62, 216 pp.
3. HICKMAN, C.J., and D. ASHWORTH. 1943. The occurrence of Botrytis spp. on onion leaves with special reference to B. squamosa. Brit. Mycol. Soc. Trans. 26: 153-157.
4. McLEAN, D. M., and BAILEY SLEETH. 1959. Tip and leaf blight on onions in the Lower Rio Grande Valley. J. Rio Grande Valley Hort. Soc. 13: 152-154.
5. PAGE, O. T. 1953. Botrytis spot of onion leaves in Ontario. Plant Disease Reptr. 37: 513-514.
6. PURDY, L. H. 1955. A broader concept of the species Sclerotinia sclerotiorum based on variability. Phytopathology 45: 421-427.
7. VIENNOT-BOURGIN, G. 1952. Sur la presence en France de Botrytis squamosa, parasite de 1 'oignon. Rev. pathol. vegetale. et entomol. agr. France 31(2): 82-98.
8. VIENNOT-BOURGIN, G. 1953. Un parasite nouveau de 1 'oignon en France: Bortytis squamosa Walker et sa forme parfaite, Botryotinia squamosa sp. nov. Annales des Epiphytes, Ser. C. Ann. 4(1): 23-43.
9. WALKER, J. C. 1925. Two undescribed species of Botrytis associated with neck rot diseases of onion bulbs. Phytopathology 15: 708-713.
10. WALKER, J. C. 1926. Botrytis neck rots of onions. J. Agr. Research 33: 893-928.
11. WHETZEL, H. H. 1945. A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. Mycologia 37: 648-714.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE,
UNITED STATES DEPARTMENT OF AGRICULTURE, WESLACO, TEXAS

BACTERIAL SPOT RESISTANCE OF INTRODUCED PEPPERS[1]

Grover Sowell, Jr.[2]

Abstract

P.I. Numbers 163184, 163189, 163192, 164471, 164677, 173877, 182646, 182925, 183439, 183440, 183441, 183922, 244669, 244670, and 246331 of Capsicum frutescens are sufficiently resistant to bacterial spot to be of value to plant breeders as sources of resistance to the disease.

REVIEW OF LITERATURE

Several reports of resistance of pepper (Capsicum frutescens) to bacterial spot, caused by Xanthomonas vesicatoria (Doidge) Dowson, occur in the literature. Horsfall and McDonnell (4) noted that several commercial varieties were free of the disease or showed only a few spots on the leaves. Among the most resistant in their tests were Bullnose, Waltham Beauty, Yellow Oshkosh, Sweet Yellow, Harris Earliest, and Harris Early Giant. Weber (8) reported considerably less disease among the "hot" varieties than among the mild or sweet varieties. Under Florida conditions he did not observe resistance in World Beater, Bullnose, or Early Giant, all of which were resistant in Horsfall and McDonnell's test. Hogan (3), on the other hand, reported that the variety World Beater was resistant in Florida. Decker and Anderson (1) reported that certain strains of pepper showed very little disease, but they did not identify these strains. Martin (6) discovered two highly resistant strains of pepper, one of the santanka (4a-2-1) and one of the cayenne (4566) type. Krupka (5) recently screened approximately 40 commercial varieties for resistance to bacterial spot. Of the varieties tested, Long Red Cayenne and Calcom were resistant. Greenleaf (2) reported that a pepper from Ceylon, named G-2 - Deva (P.I. 246331[3]) possessed a higher resistance level than any other pepper previously tested including Santanka and Mexican Wooly (W. W. Hare, Mississippi State University, 252B).

MATERIALS AND METHODS

Greenhouse Screening Tests: A series of experiments was conducted to determine the best method of growing the plants and the inoculum, of preparing and standardizing the inoculum, of inoculating and incubating the plants, and of taking the data on infection. For the sake of brevity these experiments are not described in detail. The technique described herein was selected for use. A highly pathogenic isolate of the bacterial spot organism (TS8), which was isolated by the writer from tomato at Bradenton, Florida, was used. The cultures were maintained at 15° C on potato-dextrose agar (PDA) prepared from fresh potatoes. Dehydrated PDA was not satisfactory. The bacterium was grown on agar-slant, test-tube cultures for 48 hours at room temperature (25° to 30° C). Petri-dish cultures were then prepared by flooding each test-tube culture with approximately 2 ml of sterile distilled water, suspending the bacterium by scraping the agar surface with a wire transfer loop, and pouring the resulting suspension over solidified PDA in Petri dishes. These Petri-dish cultures of the pathogen were held for 48 hours at room temperature, at which time the surface of the agar was covered by the yellow viscous mass consisting of the bacterium and its exudate. Next, a bacterial suspension was prepared by adding distilled water to the Petri-dish cultures and scraping the surface of the agar with a rubber policeman. One or two Petri-dish cultures were used per liter of distilled water. The relation between the number of viable bacterial cells per ml and the optical density (OD) of a suspension was determined with a spectrophotometer at a wave length of 400 mμ. The number of viable bacterial cells per ml was determined by a dilution technique. Suspensions prepared with one Petri-dish culture per liter of distilled water contained approximately 1×10^6 viable bacterial cells per ml and demonstrated an OD of .10. A suspension prepared with two Petri-dish cultures per liter of distilled water contains approximately 2×10^6 viable bacterial cells per ml and has an OD of .18.

---

[1] Approved by the Director of the Georgia Agricultural Experiment Stations as Journal Series Paper No. 368.

[2] Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Experiment, Georgia.

[3] Plant Introduction number.

The first group of plants to be screened for disease resistance was inoculated with a bacterial suspension adjusted to an OD of .10. The incidence of disease in this test was less than that considered as desirable, however, and the second group of plants was inoculated with a suspension prepared by suspending two Petri-dish cultures per liter of distilled water and adjusted to an OD of .18. In subsequent experiments the OD varied from .15 to .19.

The pepper seeds were planted in methyl bromide-fumigated soil to which fertilizer and peat moss had been added. Fifty seeds of each variety or plant introduction were planted in single rows 3 1/4 inches apart in flats. When the plants were approximately 30 days old they were inoculated with the bacterial spot organism.

Inoculation was accomplished with a stainless-steel knapsack sprayer. The sprayer was equipped with a pressure-control regulator set to deliver 20 to 25 pounds per square inch pressure. During inoculation the nozzle of the sprayer was held directly over the row and at a 45-degree angle to the vertical axis of the plants. The nozzle was moved slowly along the row so that maximum coverage without excess run-off was obtained.

After inoculation the plants were incubated in a moist chamber, which consisted essentially of a wooden frame covered with polyethylene plastic film. The humidity in the moist chamber was held near saturation by means of a humidifier controlled by an interval timer. The setting of the interval timer is very critical in this method of maintaining a saturated atmosphere. If it is set so that the humidifier runs more than 3 1/2 minutes of the 10-minute cycle, run-off occurs and the bacteria are washed off the plants. If, on the other hand, the setting is below 3 1/2 minutes the plants may dry off, under the weather conditions at Experiment, Georgia, and infection will be reduced or very limited. The plants were left in the moist chamber for 40 hours.

Data on the incidence and severity of bacterial spot on the plants were taken 7 to 10 days after inoculation by the method of Krupka (5). Incidence was recorded as percentage of plants infected. Severity was estimated by means of a rating scale of 0 - 3, (0 = 0 spot per leaf, 1 = 1-2 spots per leaf, 2 = 3-14 spots per leaf, and 3 = 15 or more spots per leaf). This rating of severity is referred to hereafter as the "disease index."

Replicated Greenhouse Tests: All lines exhibiting resistance (less than 30% of the seedlings infected) in the greenhouse screening tests were tested in replicated greenhouse tests. The experimental design was a randomized block with four replications. The disease-index data were analyzed as such, but the percentage of plants infected was converted to angle as recommended by Snedecor and Cochran (7) before the analysis of variance was calculated.

Replicated Field Test: In the summer of 1959, 19 lines which had exhibited resistance in greenhouse screening tests or had been reported as resistant by previous investigators were grown at Experiment. Two lines known to be susceptible were included as standards. The plots were inoculated three times with isolate TS8 of Xanthomonas vesicatoria. This experiment could not be analyzed statistically because of an inadequate supply of plants for the four replications originally planned.

RESULTS

Greenhouse Screening Tests: Of the 659 lines screened for resistance to X. vesicatoria, 32 exhibited resistance in this series of tests. Selections previously made within an introduction do not differ enough to justify considering these selections as distinct lines insofar as resistance to X. vesicatoria is concerned. Even the difference of 34.5% as shown by P.I. 163189 (Selection A) and P.I. 163189 (Selection B) is not considered significant by the writer in an unreplicated test. California Wonder, the susceptible standard, was severely affected as indicated by a disease index of 3 and 100% of seedlings infected.

Replicated Greenhouse Tests: In the first replicated greenhouse test, the commercial variety Anaheim, P.I. 164471, P.I. 164677, and a selection from P.I. 163189 were superior in resistance, as evaluated by disease index, to all other lines tested (Table 1). The data on percentage infection in Test Number 1 in Table 1 indicate that the lines with a low disease index also exhibited a low incidence of disease when this second method of evaluation was used. P.I. 163184 and P.I. 163192 also appeared to have a significant amount of resistance. World Beater, which has been previously reported as resistant, was superior to all other commercial varieties of sweet pepper tested. Improved Truhart Pimiento was significantly superior to California Wonder when resistance was evaluated by disease index. In the second replicated greenhouse test, the severity of bacterial spot as measured by disease index was much less than in the preceding test (Table 1) and statistically significant differences were not obtained. P. I. 173877, P.I. 182646, P.I. 182925, P.I. 183439, P.I. 183440, P.I. 183441, P.I. 183922, P.I.

244669, P.I. 244670, P.I. 246331, and selection 158 (from J. A. Martin, Clemson College, Clemson, South Carolina) were more resistant than the susceptible standards California Wonder and Improved Truhart Perfection by highly significant amounts when disease severity was evaluated by percentage infected plants. Anaheim, which appeared highly resistant in Test Number 1, did not demonstrate resistance in Test Number 2.

Table 1. The severity of bacterial spot on selected introductions and varieties inoculated with Xanthomonas vesicatoria in replicated greenhouse tests.

P.I. number or variety	Average disease severity		
	Index	%	Angle of %
Test Number 1			
163,184	1.00	35.1	36.4
163,189	.75	22.3	28.2
163,192	1.00	35.3	36.4
164,471	.75	18.8	25.7
164,677	.75	29.0	32.5
Anaheim	.75	15.7	23.3
California Wonder	1.75	74.9	59.9
Improved Truhart Pimiento	1.25	88.9	70.5
World beater	1.00	70.5	57.1
L.S.D. 0.05	.14		18.91
L.S.D. 0.01	.18		25.18
Test Number 2			
173,877	1.00	35.4	36.5
182,646	1.00	40.3	39.4
182,925	1.00	38.8	38.5
183,439	1.00	14.8	22.6
183,440	1.00	32.1	34.5
183,441	1.00	25.6	30.4
183,922	1.00	32.9	35.0
244,669	1.00	33.9	35.6
244,670	1.00	18.4	25.4
246,331	1.00	15.3	23.0
Anaheim	1.00	64.8	53.6
California Wonder	1.00	95.5	77.8
Improved Truhart Pimiento	1.00	76.1	60.7
158 (J. A. Martin)	1.00	39.3	38.8
L.S.D. 0.05	.67		14.51
L.S.D. 0.01	.87		19.32

Replicated Field Test: P.I. numbers 125807, 163184, 163189, 163192, 164471, 164677, 246331, and selection 158 showed less than 50% infected seedlings and an average infection index of 1 or less. California Wonder and Improved Truhart Pimiento showed 100 and 95% infection, respectively, and an average infection index of 2.0 and 1.3.

DISCUSSION

The only source of resistance reported by previous investigators which appears to be equal to that of the resistant lines discovered in the present investigation is that of P.I. 246331 supplied by W. H. Greenleaf (2). The wide variation in resistance shown by the variety Anaheim in successive replicated tests indicates that the resistant lines should be evaluated more thoroughly. Such an evaluation would enable the plant breeder to select the most highly resistant line for use as a source of resistance.

Most of the resistant introductions have small, pungent fruits. P.I. 182925 is outstanding, however, since individual plants have been observed to produce large, sweet fruits. This may permit the plant breeder to obtain a pepper with desirable fruit characteristics by making a minimum number of back-crosses to the susceptible parent.

A mimeographed report giving the data from each experiment in greater detail is being prepared and will be available on request.

Literature Cited

1. DECKER, PHARES, and C. W. ANDERSON. 1953. The resistance of peppers, Capsicum frutescens L., to virus diseases. Florida Agr. Exp. Sta. Ann. Rept. 1953: 129-130.
2. GREENLEAF, W. H. 1960. Resistance levels to the bacterial leaf spot disease in peppers. (Abst.) Proc. Assoc. Sou. Agr. Workers. 57th Annual Conv. 1960. (In press)
3. HOGAN, G. B. 1942. Blight resistant - World Beater pepper. Proc. Florida State Hort. Soc. 55: 149.
4. HORSFALL, J. G., and A. D. McDONNELL. 1940. Variety susceptibility of peppers to bacterial spot. Plant Disease Reptr. 24: 34-36.
5. KRUPKA, L. R. 1956. The life cycle, physiology and control of Xanthomonas vesicatoria (Doidge) Dowson. University of Delaware, Master of Science Thesis.
6. MARTIN, J. A. 1948. Bacterial spot resistance in peppers. Proc. Am. Soc. Hort. Sci. 52: 336-342.
7. SNEDECOR, G. W., and W. G. COCHRAN. 1956. Statistical methods applied to experiments in agriculture and biology. Iowa State College Press, Ames, Iowa.
8. WEBER, G. F. 1932. Diseases of peppers in Florida. Florida Agr. Exp. Sta. Bull. 244: 6-9.

REGIONAL PLANT INTRODUCTION STATION, EXPERIMENT, GEORGIA

MODIFICATION AND ADAPTATION OF POPP'S TECHNIQUE TO ROUTINE DETECTION OF USTILAGO NUDA (JENS.) ROSTR. IN BARLEY EMBRYOS[1]

T. Kavanagh and D. L. Mumford[2]

A program for testing samples of seed barley for loose smut was established at St. Paul, Minnesota in the fall of 1959. Popp's[3] method of detecting Ustilago nuda mycelium in whole embryos was modified and adapted to the requirements of testing a large number of samples. The following procedure has been used successfully in testing more than 600 samples.

Approximately 500 grains from a thoroughly mixed barley sample were placed in a 1-liter Erlenmeyer flask containing 250 ml of solution A (described below) and autoclaved for 45 minutes at 7 pounds' pressure. After autoclaving, the embryos were separated from the rest of the grain material in two steps: a) Two intermeshing sieves (U. S. Standard No. 8 and No. 16) partially submerged in water were used to separate the embryos from the bulk of the debris. The upper sieve (No. 8) allowed the embryos to pass through but retained larger material. The embryos were collected on the lower sieve (No. 16). b) The embryos were further freed from the remaining hulls and smaller particles by transferring them in water to a 5-inch high 250-ml jar and adding sufficient concentrated sodium silicate to float the embryos to the top of the liquid, leaving the hulls and other debris at the bottom. Approximately 50 ml of sodium silicate added to 200 ml water accomplished this separation. The embryos were poured off from this jar into a 250-ml Erlenmeyer flask.

The embryos were cleared and stained as described by Popp, but the following procedures resulted in the saving of time and chemicals:

After pouring off the water from the embryos, 50 ml of solution B was added and the flasks then autoclaved for 45 minutes at 7 pounds' pressure. After two or three washings in distilled water, the embryos were transferred to Gooch crucibles in which they were retained through the remaining steps of the process until they were ready for microscopic examination. These steps were as follows: The crucibles containing the embryos were placed in about 20 ml of solution C in 50-ml beakers and autoclaved for 1 minute after the temperature had reached 100° C at 7 pounds' pressure. The embryos within the crucibles were similarly autoclaved for 1 minute successively in solution D, solution E, and solution D in 50-ml beakers. The efficiency of these steps was greatly increased by processing a large number of samples at one time.

For microscopic examination, solution D was drained off and the embryos were transferred in water to a Lucite tray with parallel ridges which divided the tray into a number of channels, connected with one another alternately at the right and left hand sides. In this way, a continuous channel was formed from the top left to the bottom right hand side of the tray. This arrangement reduced the possibility of omitting one line or counting one line twice when the embryos were being examined under a stereoscopic binocular microscope at 20X.

The solutions indicated by letter above were prepared as follows:

Solution A -- Mix equal parts by volume of 6% sodium hydroxide and 24% sodium silicate. To each liter of this solution add 0.4 ml of Tween 20. Solution B -- Mix equal parts by volume of 24% ethanol and 30% sodium hydroxide (Technical flakes). Solution C -- Mix by volume 3 parts absolute ethanol and 1 part glacial acetic acid. Solution D -- 45% lactic acid. Solution E -- 45% glacial acetic acid containing 0.1% trypan blue.

Certain advantages of the above procedure might be mentioned. The necessity of using a blender to dehull the grain was avoided by autoclaving for 45 minutes at 7 pounds' pressure to extract the embryos instead of 30 minutes at 5 pounds' pressure as recommended in the original method. The use of intermeshing sieves partially submerged in a pan of water permitted rapid separation of the embryos without damaging them. Floating the embryos off by the use of sodium silicate removed the remaining hulls and debris which, if present in a sample, increased the time required for counting. The use of Gooch crucibles enabled the operator to transfer the embryos rapidly from one solution to another, including the opaque trypan blue stain, without the necessity of devoting much time and care to their retention and recovery. Although, with different clearing procedures, the infected embryos can be counted without staining, staining increased the ease and accuracy of counting. Supporting the crucibles in 50 ml beakers made it possible to treat the embryos in only small amounts of the various solutions. The design of the Lucite tray was such that the possibility of error in counting was reduced to a minimum.

In the routine use of the above procedure it was possible to obtain an average yield of 95% of the embryos for final reading. When one technician was testing groups of at least 10 samples the time requirement was about 40 minutes per sample. This time can be reduced further if high yields of embryos are not sought.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY, INSTITUTE OF AGRICULTURE, UNIVERSITY OF MINNESOTA, ST. PAUL, MINNESOTA

[1]Paper No. 4374, Scientific Journal Series, Minnesota Agricultural Experiment Station.
Supported in part by a grant from the Malting Barley Improvement Association, Milwaukee, Wis.

[2]Graduate student and Research Fellow, respectively, Department of Plant Pathology and Botany, University of Minnesota.

[3]Popp, W. 1958. An improved method of detecting loose smut mycelium in whole embryos of wheat and barley. Phytopathology 48: 641-643.

AN EASILY CONSTRUCTED CLIP-TYPE INSECT CAGE[1]

James W. Guthrie and Guy W. Bishop[2]

A small, light weight, easily attached cage of simple construction for confining aphids used in virus-vector experimentation on potatoes is described. This cage reduces chances of accidental infections in the field since the aphids are introduced into the cage before it is taken to the field.

The cage is constructed from a polyethylene container available in several sizes through the Turtox Company[3]. A container of 8-dram capacity was found to yield a cage large enough for satisfactory use on the rough leaf of potato. The essential parts and construction of the cage are shown in Figure 1. The body of the cage is formed by cutting off the top of the polyethylene

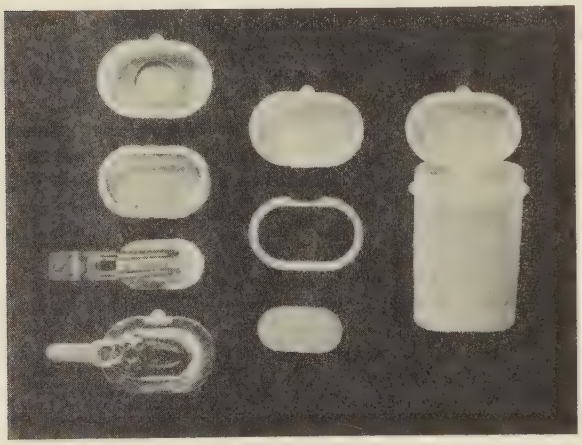


FIGURE 1. Basic materials and steps in making the clip-type insect cage.

FIGURE 2. Clip-type cage attached to a potato leaf.

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 491.

[2] Assistant Plant Pathologist and Assistant Entomologist, respectively, University of Idaho Branch Experiment Station, Aberdeen, Idaho.

[3] Turtox Biological Supplies, Catalogue 61, #315A445, 1959.

container just below the protruding lip to form a broad base. The lower surface is roughened with sandpaper and covered with nylon cloth through which the aphids feed. Cloth of about 100 mesh is recommended since the openings are small enough to prevent the escape of new born aphids but large enough to allow development. Any good waterproof glue may be used to attach the cloth to the polyethylene but the glue should be used sparingly in order to keep the surface smooth and even. The lid of the cage is made from the lid of the polyethylene container. A hole is bored through the lid for ventilation and covered on the upper surface with the nylon cloth. The body and lid when united form the basic unit of the cage. This basic unit is attached to the leaf with a spring-type hair clip of the type used by MacGillivray and Anderson (1) and Noble (2). An oval piece of plastic with dimensions slightly smaller than the nylon covered opening in the cage body is cut from the bottom of the original container. This piece is glued to one prong of the clip.

The basic unit is placed with the nylon cloth in contact with the lower surface of the leaf. The uncovered prong of the clip fits over the cage lid and the oval piece of polyethylene on the other prong fits on the upper surface of the leaf directly above the nylon mesh through which the aphids feed (Fig. 2). Tension of the spring should be sufficient to force the leaf surface against the nylon mesh. Tension may be adjusted by bending the prongs of the clip. An aluminum clip is most satisfactory since it can be easily bent to fit the conformation of the cage.

The cage has been used on potato leaves with the green peach aphid, Myzus persicae Sulz. and the cotton aphid, Aphis gossypii Glov. When a colony is kept within a cage for long periods it is necessary to move the cage at about 10-day intervals to provide a new leaf surface.

Literature Cited

1. MacGILLIVRAY, M. E., and G. B. ANDERSON. 1957. Three useful insect cages. Can. Ent. 89: 43-46.
2. NOBLE, M. D. 1958. A simplified cage for aphid investigations. Can. Ent. 90: 760.

UNIVERSITY OF IDAHO BRANCH EXPERIMENT STATION,
ABERDEEN, IDAHO

ANOTHER SCREENED IMMERSION PLATE FOR ISOLATING FUNGI FROM SOIL[1]

Francis A. Wood and Roy D. Wilcoxson[2]

The screened immersion plate technique developed by Thornton[3] is excellent for isolating fungi from soil. The principal disadvantage of the technique is the great amount of time and effort spent in preparing the apparatus for use. Therefore, a modification was developed which utilized easily assembled materials readily available in most biological laboratories.

Plates were assembled with Petri dishes and plastic discs[4], 8.5 cm in diameter, 1 mm thick, with 12 holes each 2 mm in diameter. The discs were constructed with a band saw and drill press. Discs were sterilized by dipping them into 95% ethyl alcohol and then flaming, or by autoclaving 20 minutes at 15 pounds' pressure. About 25 cc of sterile water agar were poured into Petri dishes, the sterile discs were placed on the agar surfaces, and the plates covered with the Petri dish covers until used (Fig. 1).

To isolate fungi from soil, the covers were removed and the plates pressed against the soil profile so that soil and disc were in contact. When the plates were in place they were covered with a piece of aluminum foil and then with soil. The aluminum foil helped keep unwanted soil particles out of the plates when they were removed from the soil.

After being in contact with soil 2 or more days, depending on the amount of soil moisture, the plates were removed and the fungi were isolated. In the laboratory the position of the holes in the disc was marked on the bottom of the Petri dish with a wax pencil and then the disc was removed. The agar over the marked areas was examined immediately and the fungi were isolated by transferring hyphae to other dishes. A dissecting microscope and indirect lighting were used for this work.

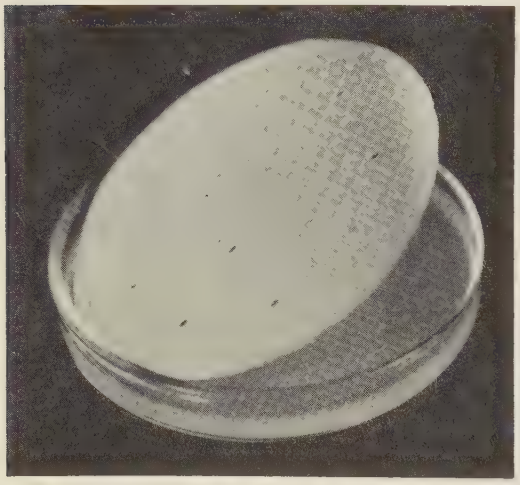


FIGURE 1. A modified screened immersion plate.

Using this technique, species of the following genera were isolated: Candida, Fusarium, Glenospora, Monilia, Papulospora, Phialophora, Phoma, and Rhizoctonia. In addition, other fungi, which did not sporulate, were isolated and were arbitrarily placed into ten groups according to distinct mycelial characteristics. Dilution plate isolations from the same soil yielded Aspergillus candidus Link, A. niger Van Tieghem and species of Acremonium, Botrytis, Cephalosporium, Chaetomium, Cunninghamella, Fusarium, Fusidium, Hormodendrum, Penicillium, Phoma, Rhizopus, Stysanus, Trichoderma, and Verticillium. Only species of Fusarium and Phoma were isolated with both methods. Thus, the modfied screened immersion plate favored isolation of fungi which did not sporulate, whereas dilution plates favored isolation of fungi which sporulate profusely, as shown by Chesters and Thornton[5] with the original screened immersion plate.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY, INSTITUTE OF AGRICULTURE, UNIVERSITY OF MINNESOTA, ST. PAUL, MINNESOTA

---

[1]Paper No. 4371, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2]Research Assistant and Assistant Professor, respectively, Department of Plant Pathology and Botany, University of Minnesota.

[3]Thornton, R. H. 1952. The screened immersion plate: A method of isolating soil microorganisms. Research 5:190-191.

[4]A fiber-glass plastic product of Filon Plastics Co., 333 N. Vaness, Hawthorne, California.

[5]Chesters, C. G. S., and R. H. Thornton. 1956. A comparison of techniques for isolating soil fungi. Trans. Brit. Mycol. Soc. 39:301-313.

POWDERY MILDEW OF POTATO IN ARGENTINA

R. E. Pontis and J. M. Feldman[1]

During the month of March 1960 shoots of maturing potato plants of the variety Up-to-date, which were growing in a field at the high valley of Las Carreras (Mendoza), were observed to be infected by a white sparse extramatrical mycelium, suggesting a powdery mildew.

Microscopical observations showed conidiophores and conidia in chains, typical of the imperfect genus Oidium. Measurements on 100 mature conidia gave an average size of 29.15 μ long by 16.46 μ wide, and varied from 24 μ to 36 μ long and 13.2 μ to 21.6 μ wide; this is about the same average reported by Menzies[2]. As the writers have not found perithecia, this fungus cannot be positively named yet.

The most usual site of infection, as in Washington State[2], is the leaf petioles and along the stem. We have not found the fungus on the leaves, as has been reported in Palestine[3] and England[4].

Examination of commercial crops of other varieties has not revealed a general occurrence of the disease and there seems to be no commercial damage to potato from this parasite. Owing to the inconspicuous development of powdery mildew, it is quite possible that the fungus could have been present, but undetected, for many years. However, this appears to be the first report of a powdery mildew on potatoes in Argentina.

INSTITUTO DE SANIDAD VEGETAL, FACULTAD DE CIENCIAS AGRARIAS,
UNIVERSIDAD NACIONAL DE CUYO, MENDOZA, ARGENTINA

---

[1]Professor of Plant Pathology and Instructor in Plant Pathology, respectively.

[2]Menzies, J. D. 1950. Erysiphe cichoracearum DC. as a parasite of potatoes. Plant Disease Reptr. 34: 140-141.

[3]Chorin, M. 1946. The powdery mildews of potatoes in Palestine. Palestine J. Botany (Rehovot Ser.), 5(2): 259-261.

[4]Thomas, D. G. 1946. Powdery mildew of potato. Nature (London), 158: 417-418.

A HIGH INCIDENCE OF DIAPORTHE PHASEOLORUM OCCURRING IN THE SEED OF SOYBEANS FROM SOUTHWESTERN ONTARIO[1]

V. R. Wallen[2]

Soybean seed of many varieties including Harosoy, Hardome, Chippewa, Harmon, Lincoln, Comet, Blackhawk, Mandarin, Merit and Hawkeye have been found to be internally infected with the fungus Diaporthe phaseolorum (Cke. & Ell.) Sacc. in 1959.

Samples[3] of seed were collected from representative areas in Kent, Essex, Lambton, Middlesex, and Elgin counties. Of 150 samples examined for the fungus, over 90% were infected. In the various lots, 1 to 87% of the seed was infected and the average infection was 28.88%.

In greenhouse trials with non-sterile soil (two parts loam, one part peat moss and one part sand) the average emergence of the samples was 77.36%, 10.56% of which seedlings showed aboveground symptoms of the disease. The average emergence of healthy seed lots was 91.8%.

Although it has occurred only sporadically in previous years, the disease has reached epiphytotic proportions this year. The extremely hot humid weather that prevailed during the latter part of the growing season in 1959 probably induced early maturity of the soybean crop. The fungus normally attacks late in the season and does not have sufficient time to establish itself within the seed.

PLANT RESEARCH INSTITUTE, CANADA DEPARTMENT OF AGRICULTURE, OTTAWA, ONTARIO

---

[1]Contribution No. 66, Plant Research Institute, Canada Department of Agriculture, Ottawa, Ontario.
[2]Plant Pathologist, Plant Research Institute, Ottawa, Ontario.
[3]Samples submitted by Dr. T. F. Cuddy, Plant Products Division, Canada Department of Agriculture, Ottawa, Ontario.

A NEW RECORD OF VIOLET SCAB (SPHACELOMA VIOLAE)

Anna E. Jenkins

In New York State we have the record of violet scab (Sphaceloma violae Jenkins) on Viola odorata grown in the field at Rhinebeck, Dutchess Co. (1, p. 3) and on Viola spp. in a garden at Forest Home, Tompkins Co. (2). Still another locality in Dutchess Co. is that of Hopewell Junction. There, on September 17, 1959, I discovered the disease in abundance on Viola sp. (blue-flowered) growing spontaneously in front of a homestead. Representative specimens are deposited in the National Fungus Collections.

Literature Cited

1. MASSEY, L. M. and A. E. JENKINS. 1935. Scab of violet caused by Sphaceloma. New York (Cornell) Agr. Exp. Sta. Mem. 176. 9 pp.
2. WHETZEL, H. H. 1944. The scab disease of violets. Plant Disease Reptr. 28: 769-770.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

BOOK REVIEW

"DISEASES AND PESTS OF ORNAMENTAL PLANTS" (Third Edition), by Pascal P. Pirone, Bernard O. Dodge, and Harold W. Rickett. The Ronald Press Company, New York. x + 775 pages. 1960. Price $10.

Readers familiar with earlier editions of "Diseases and Pests of Ornamental Plants" will find that the third edition differs primarily in recommendations for the chemical control of diseases and pests. The first-time reader, be he scientist, nurseryman, or dedicated gardener, will be surprised by and pleased with the quality and extent of information on diseases and pests of ornamentals and their control. The book is the ultimate handbook for anyone interested in its topic.

The authors, noted scientists of the New York Botanical Garden, not only have applied their own knowledge and experience to the development of this volume, but have used many other authoritative sources. They have drawn from such scientists as Cynthia Westcott, A. W. Dimock, E. P. Felt, W. H. Rankin, Arnold Mallis, and W. R. Eadie and from such organizations and institutions as the Entomological Society of America, the U. S. Department of Agriculture, and many state universities.

"Diseases and Pests of Ornamental Plants" is divided into two parts: the first concerned with a general discussion of plant diseases, insects and other animal pests, and their control; the second, with diseases and pests of particular hosts and what to do about them. Part I discusses rusts, smuts, mites, fumigants, and mist blowers, for example. Part II, in the section on "Rhododendron", describes symptoms and gives specific control recommendations for such diseases as crown-rot and leaf-spots; for insect pests such as scales and white-flies. Part II, which extends from Abelia to Zinnia, includes in all some 600 ornamental plants.

One can be fairly sure that a fourth edition of such a useful book as this will eventually follow the third. As scientists continue to learn more about the diseases and pests of garden plants, and as newer and more selective chemicals are introduced for their control, Part II of this volume is doomed to go out of date. There has been a virtual revolution in chemical disease and pest control between the first edition in 1943 and the second in 1948 and between the second and the third.

An unusual aspect of this book is the adult approach in handling references to the safe use of pesticidal chemicals. They are sufficient, but not belabored. The reader is told of the relative hazards of DDT, malathion, and parathion, for example. He is advised to read the label and follow the directions for use printed on chemical containers. But he is not subjected to the frightening detailed warnings featured in much current writing dealing with chemical pest control.

This edition is printed in large, readable type so that it will be usable in a garden shed as well as in a library. Disease symptoms and pests are well illustrated so that they can be easily identified. -- PAUL R. MILLER